REMARKS

Oath/Declaration

The examiner has stipulated that the oath or declaration is defective because the filing date of the provisional application is defective. The filing date of the provisional application has been corrected to read 01/17/2003 on the enclosed corrected oath and declaration.

Specification

An abstract on a separate sheet as required by 37 CFR 1.72(b) is enclosed with this amendement.

The Applicant also encloses with this amendment copies of References 41-43, 45, and 48-50 as requested by the Examiner.

The Applicants believe that all of the objections have been overcome by this supplemental amendment, and they therefore earnestly solicit an early Notice of Allowance.

Respectfully submitted,

By Philippe L. Durette

Philippe L. Durette
Reg. No. 35,125
Attorney for Applicants
Merck & Co., Inc.
P.O. Box 2000
Rahway, NJ 07065-0907
(732) 594-4568

Date: May 4, 2007
Enclosures

Expert Opinion

1. Introduction
2. Dipeptidyl peptidase IV inhibitors as new oral antidiabetic drugs
3. What may we regard as the key properties of dipeptidyl peptidase IV inhibitors?
4. How do dipeptidyl peptidase IV inhibitors compare with glucagon-like peptide-1 analogues?
5. Other indications for dipeptidyl peptidase IV inhibitors
6. Expert opinion and future perspectives

Monthly Focus: Endocrine & Metabolic

Inhibitors of dipeptidyl peptidase IV: a novel approach for the prevention and treatment of Type 2 diabetes?

Carolyn F Deacon†, Bo Ahrén & Jens J Holst
†Department of Medical Physiology, Panum Institute, University of Copenhagen, DK-2200 Copenhagen N, Denmark

Inhibitors of the enzyme dipeptidyl peptidase IV (DPP IV) are of increasing interest to both diabetologists and the pharmaceutical industry alike, as they may become established as the next member of the oral antidiabetic class of therapeutic agents, designed to lower blood glucose and, possibly, prevent the progressive impairment of glucose metabolism in patients with impaired glucose tolerance and Type 2 diabetes. DPP IV has become a focus of attention for drug design, as it has a pivotal role in the rapid degradation of at least two of the hormones released during food ingestion, a property that has warranted the design of inhibitor-based drugs. At the molecular level, DPP IV cleaves two amino acids from the N-terminus of the intact, biologically active forms of both so-called incretin hormones, glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide (formerly known as gastric inhibitory polypeptide), resulting in truncated metabolites, which are largely inactive. Inhibition of the enzyme, therefore, is thought to increase levels of the active forms of both incretin hormones, culminating in an increase in insulin release after a meal, in a fully glucose-dependant manner. DPP IV inhibitors combine several features of interest to the drug design process. They can be readily optimised for their target and be designed as low molecular weight, orally active entities compatible with once-daily administration.

Keywords: dipeptidyl peptidase, enteroinsular axis, enzyme inhibitor, glucagon-like peptide-1, glucose-dependent insulinotropic polypeptide, incretin, oral antidiabetic agent

Expert Opin. Investig. Drugs (2004) 13(9):1091-1102

1. Introduction

Type 2 diabetes is a component of a disease cluster collectively known as the metabolic syndrome, comprising of a variety of disorders including glucose intolerance/insulin resistance, arterial hypertension, dyslipidaemia and obesity. It has emerged as one of the world's major debilitating diseases. A clear requirement for new and more effective drugs for the prevention and treatment of Type 2 diabetes is demonstrated by a combination of the following points:

- the well-reported escalation in the numbers of people suffering from this disease cluster
- an overtly unsatisfactory, and still surprisingly small, family of currently available treatments, the outcome of which leaves many patients with a reduced quality of life because of associated complications including cardiovascular problems, retinopathy, nephropathy and neuropathy
- a lack of available drugs for the prevention of the condition.

Almost all of today's drug therapies for Type 2 diabetes have been a result of the serendipitous discovery of the antidiabetic activity of compounds whose precise mechanisms of action were obscure at the time of discovery and, in some cases, remain unresolved. This means that many of the currently available drugs have some clinically relevant side effects, which may have been avoided had rational drug design been possible. This picture may well now be set to change due to the emergence of a new treatment modality that has resulted from the rational design of a drug class based on the precise knowledge of the salient molecular target. It is not often that clinical proof of concept (CPOC) of an entirely new oral treatment modality emerges for a major debilitating disease. The inhibitors of dipeptidyl peptidase IV (DPP IV) for the treatment of Type 2 diabetes do, however, represent such a concept.

2. Dipeptidyl peptidase IV inhibitors as new oral antidiabetic drugs

Following promising results in preclinical studies, mainly in rodents, Ahrén and colleagues [1,2] reported CPOC for the use of selective and specific inhibitors of DPP IV to treat Type 2 diabetes. This enzyme is responsible for the rapid degradation of the body's so-called incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are integral components in the physiological control of insulin release and, therefore, in the regulation of blood glucose. Consequently, the inhibition of this enzyme will prevent the degradation of these important incretin hormones, leading to enhancement of their physiological effects.

2.1 Background

In the late 1980s, the newly discovered gastrointestinal hormone GLP-1 [3,4] was found to possess potent insulin-releasing abilities [4-7], spurring interest in using this peptide therapeutically to treat diabetic hyperglycaemia. In addition to its insulinotropic action, GLP-1 possesses a spectrum of activities (β-cell tropic/anti-apoptotic, glucagonostatic, appetite suppressing, gastric emptying rate reducing [8]), which makes it an apparently ideal antidiabetic agent. In the early 1990s, the first reports of its effects in patients with Type 2 diabetes appeared [9,10]. Remarkably, continuous infusion of GLP-1 normalised glucose levels (both fasting and postprandial) in these individuals [11-13], even in those with poorly controlled diabetes long after sulfonylurea secondary failure [14]. Single subcutaneous injections were, however, less effective and glucose concentrations were not normalised [15,16], whereas the effects of continuous subcutaneous infusion for 6 weeks in diabetic patients has subsequently been reported with very promising results [17]. Furthermore, buccal administration of the peptide showed it to be efficient in healthy subjects and in patients with Type 2 diabetes [18,19]. The surprising and unexpected ineffectiveness of a single administration of GLP-1 turned out to be due to a rapid inactivation of the peptide *in vivo*. An earlier published meeting abstract had indicated that GLP-1 could be N-terminally degraded by plasma *in vitro* [20], and subsequently, DPP IV was shown to be capable of mediating such cleavage *in vitro* [21]. Later studies suggested that DPP IV was likely to play a major role in regulating the metabolic fate of GLP-1 *in vivo* [22-24], and it was also reported that DPP IV mediates the inactivation of GIP [24,25]. These observations were confirmed and extended to include the endogenous peptide in studies in which the enzyme activity was selectively deleted [25-28].

The principle of harnessing the endogenous incretin hormones to treat Type 2 diabetes by using DPP IV inhibitors was first described in 1995 [23]. The rationale is that by inhibiting DPP IV activity, levels of the intact, biologically active forms of both hormones will be increased into the range shown to be therapeutically useful [29]. The beauty of this approach is that the body's own normal homeostatic mechanisms are enhanced. Thus, the inhibitor can be given and will result in increased levels of incretin hormones and, therefore, insulin will be released only when the body needs it (i.e., mainly in relation to food intake because the incretin hormones are released in response to the presence of nutrients in the small intestine) [3]. Moreover, because the insulin-releasing effects of the incretins are glucose-dependent, insulin secretion is only enhanced when blood glucose levels rise [30], and the risk of hypoglycaemia is minimal. The concept is also supported by results from animals with a genetic deletion of DPP IV (the CD26 knockout mouse [27]) or with a mutant, catalytically inactive DPP IV molecule (the Fischer rat [31]), which have increased active GLP-1 and probably GIP levels, and improved glucose tolerance.

2.2 Dipeptidyl peptidase IV inhibitors as antihyperglycaemic agents

A number of acute studies in animal models have exemplified the beneficial effects of DPP IV inhibitors on glucose intolerance [32-34] and the results of chronic treatment have recently appeared [35-39]. These studies confirm that the effects of DPP IV inhibitors mainly reflect the known pharmacology of GLP-1. Although a reduction in food intake and body weight was seen in response to these drugs in two rat studies [35,36] it would appear that most early reports are suggestive of little or no effect on body weight, which is in contrast to GLP-1. However, even body weight neutrality, if proven clinically, would distinguish DPP IV inhibitors from the currently available therapies, which increase body weight and probably exacerbate the vicious cycle of events comprising Type 2 diabetes. Encouragingly, the first preliminary communication of a 1-year clinical trial with the inhibitor LAF-237 given in combination with metformin reported no weight gain over the study period [40]. The preclinical studies also suggest that insulin sensitivity may be improved by chronic DPP IV inhibition, possibly as a result of chronic lowering of blood glucose and a reduction of a phenomenon called glucose toxicity rather than as a direct effect of the drug itself, reflecting one of

the observations noted in the 6-week study of GLP-1 infusion [17]. Intriguingly, CD26 knockout mice and DPP IV deficient Fischer rats are protected against diet-induced obesity and insulin resistance [41,42], suggesting that in the longer term, DPP IV inhibition may affect body-weight control and energy homeostasis. DPP IV inhibitor-mediated preservation of the body's endogenous GLP-1 may also enhance the long-known effects of GLP-1 in terms of β-cell rescue/prevention of apoptosis, indicating a use for this drug class in the prevention of Type 2 diabetes [29] and, as a related property, in the prevention of the worsening of the disease. Indeed, chronic treatment with a DPP IV inhibitor preserved islet function in diabetic mice [39] and improved β-cell survival and islet cell neogenesis in streptozotocin diabetic rats [38].

The promise held by DPP IV inhibitors appears to be seen in the first trials in patients with Type 2 diabetes [1,2]. It is particularly noteworthy that these studies show that, with a DPP IV inhibitor, lowering of both fasting and postprandial blood sugar is possible. This is important because increased fasting and postprandial blood sugar are both thought to contribute to elevated glycosylated haemoglobin (HbA1c) levels, a key variable reflecting the average glycaemic levels over several months, known to be associated with the development of debilitating complications of Type 2 diabetes.

The publication of data from studies of ≥ 3 months in length, and from more substantially diabetic patients are awaited in order to judge both efficacy and incidence of side effects that may be expected from DPP IV inhibitors because the patients in both of Ahrén's studies were still in the early stage of the disease and the treatment was given for only 4 weeks. However, there is reason to be optimistic. Preclinical studies (e.g. [35]) in diabetic rodents show that the effects of DPP IV inhibitors on glucose tolerance become more marked as the dosing period continues, so that the efficacy after 4 weeks in the human studies reported thus far may well underestimate the eventual steady-state effect of DPP IV inhibition on fasting blood glucose in particular. Nevertheless, despite the relatively short treatment period studied, a significant effect on HbA1c was seen. The starting value of 7.4% for HbA1c, as was seen in the clinical studies of DPP IV inhibition [1,2], reflects the mild diabetes of this patient cohort. This, on the other hand, means that any fall in HbA1c would be small, in spite of significant improvements in glycaemic control, so it is all the more pleasing to see a significant reduction in HbA1c (of 0.5% to 6.9%) in these studies. The effect of DPP IV inhibition is expected to become less marked as blood glucose returns to normal (the so-called glucose dependency [30]), which is important in limiting one of the most serious of all side effects of some current therapies (i.e., hypoglycaemia). Recently reported preliminary findings from longer-term (12 weeks) monotherapy with LAF-237 [43] show sustained reductions in HbA1c (from a baseline value of 8 to reach 7.4% by the end of the study), suggesting that tachyphylaxis does not develop, with those patients with higher baseline (starting) HbA1c levels showing the greatest reduction; those with a starting level between 7 and 8% fell 0.7% compared with placebo treatment, while those between 8 and 9.5% declined 1.2% relative to placebo. Furthermore, early reports from 3- and 12-month combination therapy of LAF-237 with metformin also indicate that significant reductions in HbA1c levels are maintained [40,44]. HbA1c levels in those patients taking both LAF-237 and metformin were reduced from a starting value of 7.8 to 7.2% in the first 3 months [44], and this effect was sustained during an extension of the study (HbA1c at 7.3% after 1 year) [40]. In contrast, after an initial fall, HbA1c levels began to increase in the patients taking metformin alone, so that by the end of 3 months it was back to baseline (7.9%) [44], and continued to increase in the extension period to reach 8.4% by 1 year [40]. However, treatment of even impaired glucose tolerance is important for reducing the cardiovascular consequences of the metabolic syndrome [45,46].

One issue that is often raised is the question of adverse side effects, arising either as a consequence of inhibiting the catalytic activity of a molecule that has other functions in addition to degrading regulatory peptides, or because of the effect on multiple substrates of DPP IV. Other functions of DPP IV [47] include a role in the immune system, where it has the capacity to serve as a costimulatory surface molecule influencing T-cell activity, although in this context it is uncertain whether the catalytic activity *per se* is required. Moreover, there is now known to be a family of closely related enzymes, which share DPP IV-like catalytic activity, including the recently identified DPP 8 [48] and DPP 9 [49], which may be responsible for some of the functions previously attributed to DPP IV itself. It is possible that some of the described DPP IV inhibitors (**Figure 1** and **Table 1**) may not be completely selective for DPP IV, and may influence the activity of other enzymes, such as DPP 8 and DPP 9. In this context, early data suggest that DPP IV-selective inhibitors do not affect *in vitro* T-cell activation, while a DPP 8/9 inhibitor and a nonselective inhibitor do [50], suggesting that previously reported immunological effects of some DPP IV inhibitors could have been due to their effect on DPP 8 or DPP 9, rather than on DPP IV itself. Other preliminary *in vivo* studies, reporting that selective inhibition of DPP 8/9 is associated with profound toxicities (rodents) or adverse side effects (dogs) whereas selective inhibition of DPP IV is not [51], suggest that the issue of inhibitor selectivity must be addressed. The other question relates to whether DPP IV inhibition may be problematic in terms of adverse side effects, which theoretically may be expected from inhibition of an enzyme with apparently multiple substrates, via both the accumulation of adverse substrates and the inhibition of the formation of beneficial products (reviewed by Mentlein [52] and Lambeir *et al.* [47]). On one hand, inhibition of the multiple substrates of an enzyme may contribute to efficacy (e.g., inhibitors of angiotensin-converting enzyme [ACE] possess efficacy due to the modulation of at least two substrates, angiotensin I and bradykinin). On the other hand, it is possible that changes in the concentrations of

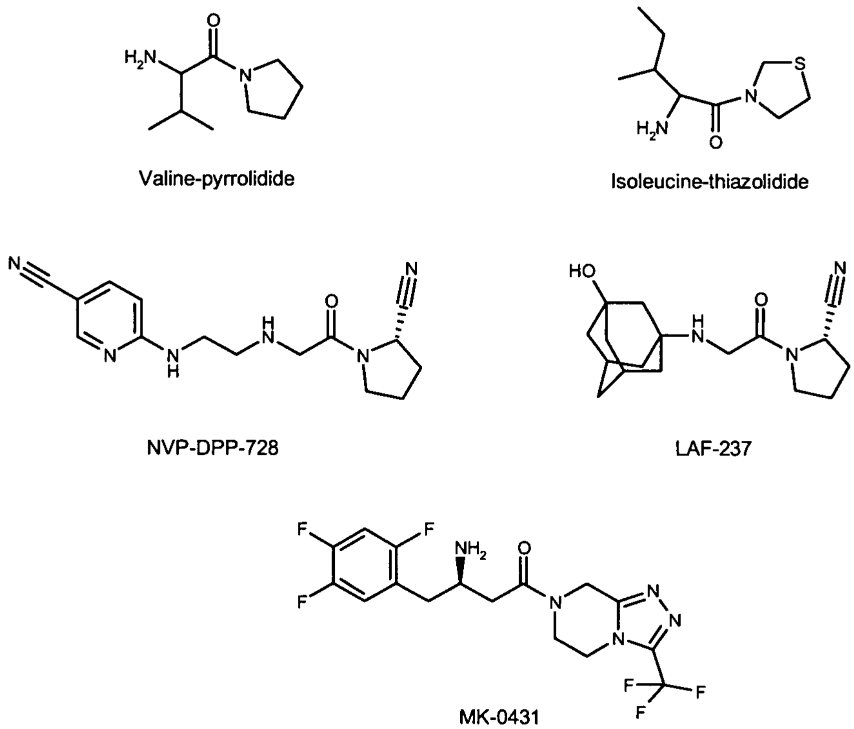


Figure 1. Structures of dipeptidyl peptidase IV inhibitors. Data are taken from [26] (valine-pyrrolidide), [32] (isoleucine-thiazolidide), [98] (NVP-DPP-728 and LAF-237) and [72] (MK-0431).

Table 1. Selectivity data for dipeptidyl peptidase IV inhibitors.

	IC_{50} / K_i (nM)					
	DPP IV	**QPP/DPP II**	**PEP**	**FAPα**	**DPP 8**	**DPP 9**
Valine-pyrrolidide	255*	26,000*	> 10,000	?	?	?
Isoleucine-thiazolidide (P32/98)	126*	7,000*	?	?	?	?
NVP-DPP-728	22	110,000	190,000	?	?	?
LAF-237	3.5	> 500,000	210,000	?	?	?
MK-0431	18	> 100,000	> 100,000	> 100,000	48,000	> 100,000

Data are taken from [97] (valine-pyrrolidide and isoleucine-thiazolidide), [98] (NVP-DPP-728 and LAF-237) and [72] (MK-0431). *These values are K_i.
DPP: Dipeptidyl peptidase; IC_{50}: Median inhibitory concentration; FAPα: Fibroblast activatoin protein-α (seprase); K_i: Inhibition constant; PEP: Prolylendopeptidase (prolyloligopeptidase/postproline cleaving enzyme); QPP: Quiescent cell proline dipeptidase.

multiple endogenous substrates may be a source of unwanted side effects. It is critically important that, when considering possible substrates for DPP IV, one differentiates between substrates that are known to be physiologically relevant (i.e., endogenous substrates) and those that have been identified in assays *in vitro* where often high concentrations of substrates are offered to the enzyme in possibly aphysiological conditions. Measurement of endogenous levels of substrates, *in vivo*, after DPP IV inhibition, provides the most relevant method for judging which substrates are relevant to the action of DPP IV inhibitors. Moreover, we should remember that even physiologically relevant substrates may also have multiple routes of degradation, so that the inhibition of DPP IV will not prevent the metabolism of these substrates via alternative routes. Ultimately, only careful assessment of efficacy versus side effects will determine whether enzyme inhibitors will become established therapy for any given indication. It is comforting to see that the incidence of pruritis, a possible consequence of substance P accumulation, was only transiently experienced, and then only in the study with the inhibitor NVP-DPP-728 [1] and not in those patients receiving LAF-237 [2], suggesting that some other DPP IV substrates may find alternative routes of degradation once DPP IV becomes inhibited for a longer period, or that this

was a compound-specific, rather than a class-specific, effect. So far, few other side effects have been reported after 4 weeks of administration of LAF-237 and NVP-728 to man [1,2], and this also seems to be the case regarding the preliminary reports on 12-week LAF-237 monotherapy [43], and 3-month and 1-year combination therapy of LAF-237 with metformin [40,44]. Currently available information suggests, therefore, that the drug class appears to be well-tolerated and those side effects reported have been minor, transient and compound, rather than class specific.

The fact that multiple substrates for DPP IV are thought to exist may also help explain the positive effects of these drugs on glycaemia. When we consider that islet function is regulated not only by substrates and incretin hormones but also by nerves [53], it can be seen how modulation of other neuropepetides may contribute to efficacy. Sympathetic, parasympathetic and sensory nerves are known to innervate the islets. These nerves not only harbour the classical neurotransmitters acetyl choline and noradrenaline but also several neuropeptides are localised to islet nerve terminals. These neuropeptides include pituitary adenylylate cyclase-activating polypeptide (PACAP), gastrin-releasing peptide (GRP) and vasoactive intestinal polypeptide (VIP) in parasympathetic nerve terminals, galanin and neuropeptide Y (NPY) in sympathetic nerve terminals and calcitonin gene-related polypeptide (CGRP) in sensory nerve terminals, all of which have been shown to affect islet function [53]. Biochemical studies have shown that several of these islet neuropeptides are substrates for DPP IV and, therefore, DPP IV inhibition may be expected to prolong their half-lifes and consequently their action. The neuropeptides shown to be substrates for DPP IV include PACAP, VIP and GRP [54,55], and for some of them at least, DPP IV seems to be relevant physiologically (e.g., PACAP [55]). It is, therefore, of relevance that a main function of these neuropeptides is to stimulate insulin secretion in a glucose-dependent manner [53,56]. Furthermore, both PACAP and GRP stimulate cellular proliferation [57-59] and inhibit apoptosis [60], whereas PACAP has also been shown to prevent the development of streptozotocin-induced diabetes in rats [61] and to reduce the hyperglycaemia in models of impaired glucose tolerance and Type 2 diabetes in rodents [62]. Therefore, an additional advantage of DPP IV inhibition could be that the islet effects of these neuropeptides are augmented, which may contribute to the beneficial effect. In fact, their antidiabetic action may explain the experience of the clinical studies undertaken so far, in that DPP IV inhibition [1,2,40,43,44] seems as efficient as GLP-1 analogues [63-66], at the doses reported, in spite of GLP-1 concentrations being increased to a lower degree than possible after analogue administration. However, it must be noted that no direct head-to-head comparisons of the maximal antihyperglycaemic effects (i.e., efficacy) have yet been reported. Furthermore, the contribution of these neuropeptides to the beneficial effect of DPP IV inhibition in relation to the contribution of GLP-1 remains to be established. After acute DPP IV inhibition, at least, all of the beneficial effects on glucose tolerance appear to be mediated via GLP-1 and GIP receptor signalling, as the glucose-lowering actions of DPP IV inhibitors were eliminated in the double incretin receptor knockout mouse, in contrast to both the single incretin receptor knockout mice, in which DPP IV inhibition does lower glucose and increase plasma insulin levels [67]. It remains to be seen whether after longer-term DPP IV inhibition, the potential neuropeptide substrates of DPP IV may contribute. Furthermore, it will only be possible to judge their contribution when analytical techniques are developed, which allow the measurement of endogenous levels of the intact and DPP IV-truncated derivatives of these neuropeptides.

3. What may we regard as the key properties of dipeptidyl peptidase IV inhibitors?

3.1 Efficacy as monotherapy: glycosylated haemoglobin lowering

It is obvious that any newly emerging oral antidiabetic (OAD) should prove effective versus placebo as monotherapy. This is difficult to judge from the published 4-week trials that are available because steady-state reductions of HbA1c are only likely to be measurable after ≥ 3 months. It should be taken into account that the reduction of HbA1c seen after 4 weeks of treatment with the inhibitors NVP-DPP-728 and LAF-237, of 0.5% [1,2], will probably end up being a significant underestimate of efficacy on a long-term basis. When judging efficiency it is important to emphasise that end-point HbA1c values correlate to the starting value and that placebo comparisons should always be made. It should also be emphasised that any reduction in HbA1c is limited when the starting value is itself low. It is, therefore, encouraging that the preliminary results after 12-week monotherapy with LAF-237 seem to suggest that, while all patients show improvements in HbA1c levels, greater reductions are achievable by those with higher starting values [43].

At present, based on the available information, it is likely that DPP IV inhibitors will have equal efficacy as monotherapy as existing OADs, and may be competitive versus the thiazolidinedione (TZD) insulin sensitisers.

3.2 Efficacy in more advanced forms of Type 2 diabetes

A long-term effect in advanced Type 2 diabetes, when insulin secretion is severely impaired, would rely on a significant effect of DPP IV inhibitors in reducing glucose excursions to a mixed meal, presumably as a result of GLP-1-induced lowering of glucagon secretion, and possibly a delay in gastric emptying. To date, there have been no clinical reports of DPP IV inhibitors affecting gastric emptying but reduced glucagon levels were reported after 4 weeks of treatment in diabetic subjects with LAF-237 [2]. Reduced glucagon concentrations would lower hepatic glucose output during the

postprandial phase. Efficacy in lowering glucagon may infer that patients may be treated even when the β-cell functional mass is reduced in more advanced cases of Type 2 diabetes, and this suggestion is supported by preclinical data. Thus, Pospisilik *et al.* [38] measured significant glucose-lowering activity in severely diabetic, streptozotocin-treated rats receiving the inhibitor P32/98 acutely and chronically and concluded that DPP IV inhibitors may be effective in subjects with Type 1 diabetes and end-stage Type 2 diabetes. In contrast, another study in diabetic *db/db* mice only found a positive effect of DPP IV inhibition in the early stage of the disease but no significant effect on glucose tolerance was observed in older animals with severe insulin resistance [68]. However, it should be noted that in the latter study, only the effect of acute DPP IV inhibition was examined. In another study in diabetic rats, the beneficial effects of DPP IV inhibition continued to improve with time, with animals showing greater improvements in glucose tolerance after 12 weeks compared with 4 weeks of treatment [35]. Therefore, more severe diabetes may require continued exposure to the inhibitor before an effect is noted. Only direct clinical investigation in patients with differing severity of the disease will show whether all patient groups will respond to DPP IV inhibitors, as has been shown for GLP-1 [14].

3.3 Effect on co-morbidities

So far, almost all treatments for Type 2 diabetes except metformin have resulted in increases in body weight, an obvious comorbidity in the disorder. The majority of data currently available indicate that DPP IV inhibitors will have no impact on body weight and thus will not suffer from the weight-increasing side effect of sulfonylureas, thiazolidinedione sensitisers and insulin. As previously mentioned, early indications from longer-term trials seem to confirm the weight-neutrality effect of DPP IV inhibitors [40]. It will now also be of considerable interest to collect data concerning the effect of DPP IV inhibitors on other relevant co-morbidities (e.g., blood pressure, other cardiovascular risk factors, plasma lipids).

3.4 Will dipeptidyl peptidase IV inhibitors become first-line therapy for Type 2 diabetes?

It may be speculated that DPP IV inhibitors will provide effective first-line therapy [29] based on a wish to treat diabetes whilst β-cell mass remains relatively healthy. We may imagine this treatment as life style changes in combination with β-cell support to begin with, followed by sensitisers in later stages of the disease.

3.5 Ability to be offered as part of combination therapy with existing therapies

It is likely that DPP IV inhibitors will combine well with metformin and TZD sensitisers because interactions between existing insulin secretagogues and insulin sensitisers is known to be encouraging and mechanistically predictable. Indeed, preclinical studies have indicated that DPP IV inhibition using the prototypal inhibitor valine-pyrrolidide, when combined with metformin, has greater effects on improving glucose tolerance, fasting blood glucose and reducing weight gain compared with either compound alone [69], while the first clinical trials seem to suggest that combining DPP IV inhibition with metformin reduces HbA1c in patients otherwise inadequately controlled with metformin alone [40,44]. Other preclinical studies indicate that the long-acting DPP IV inhibitor K-579 combines well with glibenclamide to reduce the glycaemic response to glucose loading without exacerbating hypoglycaemia [70], whereas preliminary studies suggest that a synergistic interaction between LAF-237 and pioglitazone may exist [71]. If one adds to this the glucagon suppressing effects of DPP IV inhibitors [2], the profile for combination therapy starts to look most encouraging.

3.6 Side-effect profile

Early understandable concerns that inhibiting the activity of an enzyme with multiple substrates (leading to increased concentrations of the parent substrate and reduced formation of cleavage products) will lead to problems with regard to side effects appear not to be borne out by early trials. The side-effect profile of DPP IV inhibitors, as has been ascertained so far, is encouraging.

For example, nausea and vomiting would appear not to be a problem with DPP IV inhibitors as it is for GLP-1 receptor agonists, presumably because the rise in intact, active GLP-1 observed is three- to fivefold, and therefore below the threshold for this typical GLP-1-induced side effect. Mild, transient pruritus, seen with NVP-DPP-728 [1], is likely to be compound-specific, and not due to, for example, cutaneous substance P accumulation, because LAF-237 does not seem to have this side effect [2]. Nasopharyngitis, seen with NVP-DPP-728, was only rarely observed with LAF-237. However, in preliminary communications of 3-month monotherapy with LAF-237 and the 1-year study with LAF-237 and metformin treatment, some mention was made of a modest worsening of pre-existing hypertension and mild peripheral oedema, suspected to be treatment related [40,43], underlining the need for careful monitoring of both cardiovascular function and fluid homeostasis. Moreover, it should be remembered that, in addition to potential mechanism-based side effects (i.e., relating to the selective inhibition of DPP IV), the possibility of side effects arising from non-mechanism-based actions cannot be excluded. These could include side effects caused by inhibitor nonselectivity because, as previously mentioned, inhibition of DPP 8/9 has shown profound toxicities *in vivo* [51]. At present, very little data are available regarding the selectivity of the inhibitors currently in development (Table 1), particularly with respect to DPP 8 and DPP 9, with data only being available for MK-0431 [72]. Furthermore, as the specific functions of these more recently described enzymes are still unknown, the consequences of any inhibition of their action cannot be predicted. Nevertheless, current data suggest that

LAF-237 is well-tolerated, although it should be emphasised that results from long-term studies (> 4 weeks) are not available other than as preliminary meeting reports [40,43,44].

4. How do dipeptidyl peptidase IV inhibitors compare with glucagon-like peptide-1 analogues?

At first sight, it may seem reasonable to assume that DPP IV inhibitors are simply oral GLP-1 (i.e., that they harness the potential of GLP-1 in an orally active manner). However, on more careful examination other differences between the concepts emerge. First, we consider the similarities between the two approaches.

- Glucose dependency: GLP-1 is a glucose-dependent insulin secretagogue and suppressor of glucagon, meaning that stimulation of insulin release and inhibition of glucagon will occur only at hyperglycaemic levels but not at normoglycaemic levels. In other words, the risk of hypoglycaemia as a result of either process is minimal. So far, these encouraging properties appear to be shared equally by the GLP-1 analogues and the DPP IV inhibitors.
- β-Cell mass and function: Current data suggests GLP-1 analogues and DPP IV inhibitors may share beneficial effects in terms of preserving the population of β-cells by inhibition of the rate of apoptosis and potentiation of proliferation of new β-cells [38,73].

Second, significant differences are apparent between GLP-1 analogues and DPP IV inhibitors.

- Route of administration: GLP-1 analogues are derivatives of relatively large molecular weight polypeptides. Currently known analogues are not orally available and are likely to be offered by subcutaneous injection. In contrast, DPP IV inhibitors are small, non-peptide molecules, which are likely to be formulated as tablets for oral administration.
- Pharmacokinetics: GLP-1 analogues administered subcutaneously are thought to have a pharmacokinetic profile that follows a 12- or 24-hour cycle, whereby plasma concentrations of drug relate to the time of administration of the compound together with principles of protraction (binding of the drug to albumin or intermolecular aggregations of several drug molecules). In contrast, DPP IV inhibitors are not regarded as having a primary effect *in vivo*. Rather, their effects are secondary to changes in the levels of the intact incretin hormones (and other potential substrates). Therefore, the dynamic response to DPP IV inhibitors must be as a result of enhanced levels of the intact biologically active forms of each incretin hormone, presumably reflecting an amplified version of their natural release pattern in response to meal ingestion.
- Pharmacology versus rescued endocrinology: It is obvious that plasma levels of GLP-1 analogues reflect the dose of drug administered and it is, theoretically at least, possible to inject as much as the physician determines necessary. In contrast, there is a theoretical maximum level to which hormone rescue can achieve (i.e., the rise in the intact version of each incretin can only reach 100% of the amount of total hormone in the circulation), which is, in turn, dependent upon the L-cells' secretory capacity. It is, therefore, implicit that DPP IV inhibition, in contrast to GLP-1 analogue administration, cannot raise plasma GLP-1 levels into the pharmacological range, meaning that DPP IV inhibition may not be able to take advantage of any pharmacological effects of GLP-1. In this context, exogenous GLP-1 or GLP-1 analogue administration in Type 2 diabetic patients either alone [17] or in combination with metformin [65,66] results in significant, although rather modest, weight loss, while no changes in weight are observed after DPP IV inhibitor administration [40], suggesting that the DPP IV inhibitor-mediated preservation of endogenous GLP-1 alone is insufficient to have an impact on body weight. Moreover, recent preliminary preclinical data has suggested that exogenous GLP-1 can reduce the severity of myocardial infarction in rats independently of changes in insulin or glucose concentrations, whereas DPP IV inhibition alone does not have this effect, perhaps suggesting that pharmacological concentrations of GLP-1 may be necessary to achieve this cardioprotection [74].
- Effect on intact levels of GLP-1: Contrary to theoretical expectations, DPP IV inhibitors may not raise plasma concentrations of the intact, biologically active form of GLP-1 as much as expected. In healthy and Type 2 diabetic subjects intact GLP-1 makes up ~ 20 – 50% of endogenous GLP-1 immunoreactivity [22,75,76], so that one may expect levels to increase three- to fivefold following DPP IV inhibition. In clinical studies with LAF-237 [2] and MK-0431 [77], approximately twofold increases in intact GLP-1 concentrations were reported, although it was also mentioned that there was a small decrease (~ 10%) in the total GLP-1 concentrations after MK-0431. It has been suggested that feedback of intact GLP-1 onto its own release from L-cells is responsible for this apparently contradictory observation; the phenomenon also having been observed in animal models [28,78]. Nevertheless, levels of circulating intact GLP-1 released after a meal are enhanced [2,77], as explained in Section 2.1 and in this section, and may be sufficient to explain the efficacy. However, it cannot be excluded that the change in the ratio between intact and total GLP-1 rather than the change in the level of the intact version *per se* may have an effect. In other words, DPP IV inhibition causes the proportion of circulating GLP-1 present in the intact, biologically active form, relative to total, to rise. How such a change results in insulin release and inhibition of glucagon release remains to be fully explained.
- Mediators of efficacy: Multiple mediators (e.g., GLP-1 and GIP) as previously described would seem to be responsible for the efficacy of the DPP IV inhibitors, although it cannot be excluded that inhibition of the formation of an as-yet

unidentified product may also contribute. It is apparent that multiple mediators of efficacy will result in a different pharmacodynamic spectrum of effects than that observed after injection of an analogue of one or other of the hormones involved, although it should be reiterated that the effects of acute DPP IV inhibition, at least in rodents, appear to be mediated entirely by GLP-1 and GIP [67]. However, it is uncertain to what extent preservation of intact GIP will actually contribute to the improvement of glucose tolerance in the clinic because patients with Type 2 diabetes respond poorly to GIP [79,80], in contrast to their response to GLP-1, which is preserved [9-14]. However, it has been reported that the response to GIP in diabetic patients improved following antihyperglycaemic therapy with glyburide to reduce fasting glucose levels [81]. Preclinical studies in the diabetic (Vancouver ZDF) rat show that these animals exhibit decreased pancreatic GIP receptor expression, which results in a loss of the insulinotropic response to GIP [82]. Furthermore, downregulation of the GIP receptor can be induced by exposure of INS (832/13) cells to high glucose *in vitro* and by a hyperglycaemic clamp in otherwise normoglycaemic (lean Zucker) rats, both resulting in reduced GIP receptor expression [83]. Taken together, these studies suggest that the initial effect of DPP IV inhibition in human diabetes may be mediated primarily by GLP-1 but raise the possibility that in the longer term, when glucose levels fall, the insulinotropic effect of GIP may also contribute.

Bearing in mind the relatively few similarities, and the many dissimilarities, between DPP IV inhibitors and GLP-1 analogues, it would seem to be reasonable now to regard these two approaches as entirely separable. Therefore, DPP IV inhibitors should not simply be regarded as oral GLP-1.

5. Other indications for dipeptidyl peptidase IV inhibitors

Although it is beyond the scope of this review, it is worth mentioning that DPP IV inhibitors are also being considered for other indications. Inhibition of the activity of DPP IV has been suggested as a possible therapy for rheumatoid arthritis [84,85] and neutropenia/acute anaemia [86]. In animal models, DPP IV inhibition prolonged cardiac allograft survival, suggesting a potential use in organ transplantation [87], and partially suppressed signs of experimental autoimmune encephalomyelitis, leading to the suggestion that DPP IV inhibition may be useful for the treatment of human diseases caused by T-cell-mediated autoimmune mechanisms [88]. Although some promising preclinical results have been obtained, it is worth remembering that the inhibitors used in these studies may not have been fully selective for DPP IV. It therefore cannot be excluded that some of the observed effects could have been mediated via the inhibition of other DPP IV-like enzymes, particularly given the report that both a selective DPP 8/9 inhibitor and a nonselective DPP IV inhibitor affect T-cell activation, while DPP IV-selective inhibition does not [50].

6. Expert opinion and future perspectives

The recent identification of other DPP IV-like enzymes, such as DPP 8 [48] and DPP 9 [49], together with the demonstration that inhibition of their activities is associated with severe, even lethal, side effects [51] means that the issue of inhibitor selectivity is crucial. In this respect, the determination of the three dimensional structure of human DPP IV in complex with an inhibitor (Protein Data Bank accession codes 1N1M [89], 1PGQ [90], 1NU6 and 1NU8 [91]), which allows a very detailed and focused view of the interaction between the inhibitor and the enzyme, should help with the identification of highly selective and potent DPP IV inhibitors. Looking further to the future, the researcher's mind turns to second-generation ideas and hypotheses. In this respect, it is interesting to note that a second-generation approach is already emerging. Kinetic studies have shown that GLP-1 is a substrate for the neutral endopeptidase 24.11 (NEP) [92], an enzyme that is found in high concentration in the kidney in particular [93]. Dual inhibition of NEP and DPP IV may allow the body to rescue more GLP-1 than the inhibition of DPP IV alone, and indeed, dual NEP and DPP IV inhibition has been demonstrated to give a more marked insulin release than DPP IV inhibition alone in a pig glucose challenge model [94]. Compounds with dual inhibitory activity have, recently, been reported [95]. The approach is somewhat analogous to the development of dual ACE-NEP inhibitors for the treatment of cardiovascular disorders. It will be interesting to see whether dual NEP-DPP IV inhibition results in a preferred efficacy and/or safety profile compared with conventional DPP IV inhibitors.

DPP IV inhibitors may possess antihyperglycaemic efficacy, even in more advanced forms of the disease because they not only target insulin secretion but also insulin formation and glucagon secretion. In rodents, chronic DPP IV inhibition increases islet neogenesis and β-cell survival [38], leading to improvements in both β-cell mass and function [38,96] and, therefore, this novel therapy may have the potential to modify the disease process itself, rather than simply to replace the inadequate appearance of insulin by promoting its release. Indeed, the combination of glucagon lowering with β-cell-preserving activity may even allow the use of these compounds in the treatment of Type 1 diabetes, as was suggested by data from a preclinical study in rodents [38]. Should they enter clinical practice as a new class of safe and efficacious oral antihyperglycaemic agents possessing a neutral effect on body weight (or even lowering of body weight), DPP IV inhibitors may represent a breakthrough in the treatment of Type 2 diabetes, having the potential to be effective both as monotherapy and as a component of 'stepped care' (i.e., sequential combination therapy) of Type 2 diabetic patients. Furthermore, their spectrum of effects may mean that they can be used as a preventative treatment,

targeting patients with impaired glucose tolerance or those at high-risk of developing Type 2 diabetes, to improve glucose metabolism at an earlier stage of the disease and thereby possibly reducing the prevalence of diabetic complications that afflicts so many patients. Moreover, future studies on the interactions between DPP IV inhibitors and treatments for common comorbidities, such as dyslipidaemia, obesity and hypertension, may illustrate their more general utility as part of treatment of the metabolic syndrome. Thus, optimisation of this drug class may well contribute the first mechanistic attempt to provide effective therapy of the disease by combining the lowering of glycaemia without increasing body weight, and thus help prevent the late diabetic complications that create such a profound socio-economic burden today.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. AHRÉN B, SIMONSSON E, LARSSON H *et al.*: Inhibition of dipeptidyl peptidase IV improves metabolic control over a 4-week study period in Type 2 diabetes. *Diabetes Care* (2002) 25:869-975.
   - •• **The first demonstration of a clinical effect of DPP IV inhibition in Type 2 diabetes.**
2. AHRÉN B, LANDIN-OLSSON M, JANSSON PA, SVENSSON M, HOLMES D, SCHWEIZER A: Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels and reduced glucagon levels in Type 2 diabetes. *J. Clin. Endocrinol. Metab.* (2004) 89:2078-2084.
3. ØRSKOV C, HOLST JJ, KNUHTSEN S, BALDISERRA FG, POULSEN SS, NIELSEN OV: Glucagon-like peptides GLP-1 and GLP-2, predicted products of the proglucagon gene, are secreted separately from pig small intestine but not pancreas. *Endocrinology* (1986) 119:1476-1475.
4. MOJSOV S, WEIR GC, HABENER JF: Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J. Clin. Invest.* (1987). 79:616-619.
5. HOLST JJ, ØRSKOV C, NIELSEN OV, SCHWARTZ TW: Truncated glucagon-like peptide 1, an insulin-releasing hormone from the distal gut. *FEBS Lett.* (1987) 211:169-174.
6. ØRSKOV C, HOLST JJ, NIELSEN OV: Effect of truncated glucagon-like peptide-1 [proglucagon-(78-107) amide] on endocrine secretion from pig pancreas, antrum, and nonantral stomach. *Endocrinology* (1988) 123:2009-2013.
7. FRIDOLF T, BOTTCHER G, SUNDLER F, AHRÉN B: GLP-1 and GLP-1(7-36) amide: influences on basal and stimulated insulin and glucagon secretion in the mouse. *Pancreas* (1991) 6:208-215.
8. HOLST JJ: Therapy of Type 2 diabetes mellitus based on the actions of glucagon-like peptide-1. *Diabetes Metab. Res. Rev.* (2002) 18:430-441.
9. GUTNIAK M, ØRSKOV C, HOLST JJ, AHRÉN B, EFENDIC S: Antidiabetogenic effect of glucagon-like peptide-1 (7-36)amide in normal subjects and patients with diabetes mellitus. *N. Engl. J. Med.* (1992) 326:1316-1322.
   - • **The first description of an antidiabetogenic effect of GLP-1 in Type 2 diabetes.**
10. NATHAN DM, SCHREIBER E, FOGEL H, MOJSOV S, HABENER JF: Insulinotropic action of glucagonlike peptide-I (7-37) in diabetic and nondiabetic subjects. *Diabetes Care* (1992) 15:270-276.
11. NAUCK MA, KLEINE N, ØRSKOV C, HOLST JJ, WILLMS B, CREUTZFELDT C: Normalization of fasting hyperglycemia by exogenous GLP-1 (7-36 amide) in Type 2 diabetic patients. *Diabetologia* (1993) 36:741-744.
   - •• **An important study demonstrating that GLP-1 can normalise fasting glucose concentrations in diabetic patients.**
12. RACHMAN J, GRIBBLE FM, BARROW BA, LEVY JC, BUCHANAN KD, TURNER RC: Normalization of insulin responses to glucose by overnight infusion of glucagon-like peptide 1 /7-36) amide in patients with NIDDM. *Diabetes* (1996) 45:1524-1530.
13. LARSEN J, HYLLEBERG B, NG K, DAMSBO P: Glucagon-like peptide-1 infusion must be maintained for 24 h/day to obtain acceptable glycemia in Type 2 diabetic patients who are poorly controlled on sulphonylurea treatment. *Diabetes Care* (2001) 24:1416-1421.
14. NAUCK MA, SAUERWALD A, RITZEL R, HOLST JJ, SCHMIEGEL W: Influence of glucagon-like peptide 1 on fasting glycemia inType 2 diabetic patients treated with insulin after sulfonylurea secondary failure. *Diabetes Care* (1998) 21:1925-1931.
15. NAUCK MA, WOLLSCHLÄGER D, WERNER J *et al.*: Effects of subcutaneous glucagon-like peptide 1 (GLP-1 [7-36 amide]) in patients with NIDDM. *Diabetologia* (1996) 39:1546-1553.
16. JUNTTI-BERGGREN L, PIGON J, KARPE F *et al.*: The antidiabetogenic effect of GLP-1 is maintained during a 7-day treatment period and improves diabetic dyslipoproteinemia in NIDDM patients. *Diabetes Care* (1996) 19:1200-1206.
17. ZANDER M, MADSBAD S, MADSEN JL, HOLST JJ: Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and β-cell function in Type 2 diabetes: a parallel-group study. *Lancet* (2002) 359:824-830.
   - •• **First demonstration of a long-term effect of GLP-1 in patients with Type 2 diabetes.**
18. GUTNIAK MK, LARSSON H, HEIBER SJ, JUNESKANS OT, HOLST JJ, AHRÉN B: Potential therapeutic levels of glucagon-like peptide-1 achieved in humans by a buccal tablet. *Diabetes Care* (1996) 19:843-848.
19. GUTNIAK MK, LARSSON H, SANDERS SW, JUNESKANS O, HOLST JJ, AHRÉN B: GLP-1 tablet in Type 2 diabetes in fasting and postprandial conditions. *Diabetes Care* (1997) 20:1874-1879.
20. BUCKLEY DI, LUNDQUIST P: Analysis of the degradation of insulinotropin [GLP-1 (7-37)] in human plasma and production of degradation resistant analogs. *Regul. Pept* (1992) 40:117 (Abstract).
21. MENTLEIN R, GALLWITZ B, SCHMIDT WE: Dipeptidyl peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1 (7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur. J. Biochem.* (1993) 214:829-835.
   - •• **A kinetic study indicating that DPP IV in plasma can cleave GLP-1.**

22. DEACON CF, JOHNSEN AH, HOLST JJ: Degradation of glucagon-like peptide-1 by human plasma *in vitro* yields an N-terminally truncated peptide which is a major endogenous metabolite *in vivo*. *J. Clin. Endocrinol. Metab.* (1995) 80:952-957.

23. DEACON CF, NAUCK MA, TOFT-NIELSEN M-B, PRIDAL L, WILLMS B, HOLST JJ: Both subcutaneously and intravenously administered glucagon-like peptide-1 are rapidly degraded from the amino-acid terminus in Type 2 diabetic patients and in healthy subjects. *Diabetes* (1995) 44:1126-1131.

•• **Demonstration that GLP-1 is degraded by DPP IV in humans, leading to the first suggestion that DPP IV inhibition may be therapeutically useful.**

24. KIEFFER TJ, McINTOSH CHS, PEDERSON RA: Degradation of glucose-dependent insulinotropic polypeptide (GIP) and truncated glucagon-like peptide 1 (tGLP-1) *in vitro* and *in vivo* by dipeptidyl peptidase IV. *Endocrinology* (1995) 136:3585-3596.

25. DEACON CF, DANIELSEN P, KLARSKOV L, OLESEN M, HOLST JJ: Dipeptidyl peptidase IV inhibition reduces the degradation and clearance of GIP and potentiates its insulinotropic and antihyperglycemic effects in anesthetized pigs. *Diabetes* (2001) 50:1588-1597.

26. DEACON CF, HUGHES TE, HOLST JJ: Dipeptidyl peptidase IV inhibition potentiates the insulinotropic effect of glucagon-like peptide-1 in anesthetized pigs. *Diabetes* (1998) 47:764-769.

•• **Demonstration that DPP IV inhibition *in vivo* is capable of preventing GLP-1 degradation.**

27. MARGUET D, BAGGIO L, KOBAYASHI T *et al.*: Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proc. Natl. Acad. Sci. USA* (2000) 97:6874-6879.

28. DEACON CF, WAMBERG S, BIE P, HUGHES TE, HOLST JJ: Preservation of active incretin hormones by inhibition of dipeptidyl peptidase IV suppresses meal-induced incretin secretion in dogs. *J. Endocrinol.* (2002) 172:355-362.

29. HOLST JJ, DEACON CF: Inhibition of the activity of dipeptidyl peptidase IV as a treatment for Type 2 diabetes. *Diabetes* (1998) 47:1663-1670.

30. WEIR GC, MOJSOV S, HENDRICK GK, HABENER JF: Glucagonlike peptide I (7-37) actions on endocrine pancreas. *Diabetes* (1989) 38:338-342.

31. NAGAKURA T, YASUDA N, YAMAZAKI K *et al.*: Improved glucose tolerance via enhanced glucose-dependent insulin secretion in dipeptidyl peptidase IV-deficient Fischer rats. *Biochem. Biophys. Res. Commun.* (2001) 284:501-506.

32. PEDERSON RA, WHITE HA, SCHLENZIG D, PAULY RP, MCINTOSH CH, DEMUTH HU: Improved glucose tolerance in Zucker fatty rats by oral administration of the dipeptidyl peptidase IV inhibitor isoleucine thiazolidide. *Diabetes* (1998) 47:1253-1258.

• **Demonstration that DPP IV inhibition *in vivo* is capable of improving glucose tolerance in rodents.**

33. BALKAN B, KWASNIK L, MISERENDINO R, HOLST JJ, LI X: Inhibition of dipeptidyl peptidase IV with NVP-DPP728 increases plasma GLP-1 (7-36 amide) concentrations and improves oral glucose tolerance in obese Zucker rats. *Diabetologia* (1999) 42:1324-1331.

34. AHRÉN B, HOLST JJ, MARTENSSON H, BALKAN B: Improved glucose tolerance and insulin secretion by inhibition of dipeptidyl peptidase IV in mice. *Eur. J. Pharmacol.* (2000) 404:239-245.

35. POSPISILIK JA, STAFFORD SG, DEMUTH HU *et al.*: Long-term treatment with the dipeptidyl peptidase IV inhibitor P32/09 causes sustained improvements in glucose tolerance, insulin sensitivity, hyperinsulinemia and β-cell responsiveness in VDF (fa/fa) Zucker rats. *Diabetes* (2002) 51:943-950.

•• **First demonstration that chronic DPP IV inhibition improves glucose tolerance in diabetic rodents.**

36. SUDRE B, BROQUA P, WHITE RB *et al.*: Chronic Inhibition of circulating dipeptidyl peptidase IV by FE 999011 delays the occurrence of diabetes in male Zucker diabetic fatty rats. *Diabetes* (2002) 51:1461-1469.

37. POSPISILIK JA, STAFFORD SG, DEMUTH HU, McINTOSH CH, PEDERSON RA: Long-term treatment with dipeptidyl peptidase IV inhibitor improves hepatic and peripheral insulin sensitivity in the VDF Zucker rat: a euglycemic-hyperinsulinemic clamp study. *Diabetes* (2002) 51:2677-2683.

38. POSPISILIK JA, MARTIN J, DOTY T *et al.*: Dipeptidyl peptidase IV inhibitor treatment stimulates beta-cell survival and islet neogenesis in streptozotocin-induced diabetic rats. *Diabetes* (2003) 52:741-750.

39. REIMER MK, HOLST JJ, AHRÉN B: Long-term inhibition of dipeptidyl peptidase IV improves glucose tolerance and preserves islet function in mice. *Eur. J. Endocrinol.* (2002) 146:717-727.

40. AHRÉN B, GOMIS R, STANDL E, MILLS D, SCHWEIZER A: Prolonged efficacy of LAF237 in patients with Type 2 diabetes (T2DM) inadequately controlled with metformin. *64th Scientific Sessions of the American Diabetes Association.* Orlando, USA (2004) Late-breaking 7-LB (Abstract).

41. CONARELLO SL, LI Z, RONAN J *et al.*: Mice lacking dipeptidyl peptidase IV are protected against obesity and insulin resistance. *Proc. Natl. Acad. Sci. USA* (2003) 100:6825-6830.

42. YASUDA N, NAGAKURA T, YAMAZAKI K, INOUE T, TANAKA I: Improvement of high fat-diet-induced insulin resistance in dipeptidyl peptidase IV-deficient Fischer rats. *Life Sci.* (2002) 71:227-238.

43. PRATLEY R, GALBREATH E: Twelve-week monotherapy with the DPP-4 inhibitor, LAF237 improves glycemic control in patients with Type 2 diabetes (T2DM). *Diabetes* (2004) 53(Suppl. 2):A83, 355-OR (Abstract).

44. AHRÉN B, GOMIS R, MILLS D, SCHWEIZER A: The DPP-4 inhibitor, LAF237, improves glycemic control in patients with Type 2 diabetes (T2DM) inadequately treated with metformin. *Diabetes* (2004) 53(Suppl. 2):A83, 354-OR (Abstract).

45. FESTA A, D'AGOSTINO R Jr, HANLEY AJ, KARTER AJ, SAAD MF, HAFFNER SM: Differences in insulin resistance in nondiabetic subjects with isolated impaired glucose tolerance or isolated impaired fasting glucose. *Diabetes* (2004) 53:1549-1555.

46. CHIASSON JL, JOSSE RG, GOMIS R, HANEFELD M, KARASIK A, LAAKSO M: Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. *JAMA* (2003) 290:486-494.

47. LAMBEIR A-M, DURINX C, SCHARPÉ S, DE MEESTER I: Dipeptidyl-peptidase IV from bench to bedside: An update on structural properties, functions, and clinical aspects of the enzyme DPPIV. *Crit. Rev. Clin. Lab. Sci.* (2003) 40:209-294.
- A good review of DPP IV.

48. ABBOTT CA, YU DM, WOOLLATT E, SUTHERLAND GR, McCAUGHAN GW, GORRELL MD: Cloning, expression and chromosomal localization of a novel human dipeptidyl peptidase (DPP) IV homolog, DPP8. *Eur. J. Biochem.* (2000) 267:6140-6150.
49. OLSEN C, WAGTMANN N: Identification and characterization of human DPP9, a novel homologue of dipeptidyl peptidase IV. *Gene* (2002) 299:185-193.
50. LEITING B, NICHOLS E, BIFTU T *et al.*: Inhibition of dipeptidyl peptidase IV does not attenuate T cell activation *in vitro*. *Diabetes* (2004) 53(Suppl. 2):A2, 6-OR (Abstract).
51. LANKAS G, LEITING B, ROY RS *et al.*: Inhibition of DPP8/9 results in toxicity in preclinical species: Potential importance of selective dipeptidyl peptidase IV inhibition for the treatment of Type 2 DM. *Diabetes* (2004) 53(Suppl. 2):A2, 7-OR (Abstract).
52. MENTLEIN R: Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. *Regul. Pept.* (1999) 85:9-24.
53. AHRÉN B: Autonomic regulation of islet hormone secretion. Implications for health and disease. *Diabetologia* (2000) 43:393-410.
54. LAMBEIR AM, DURINX C, PROOST P, VAN DAMME J, SCHARPÉ S, DE MEESTER I: Kinetic study of the processing by dipeptidyl-peptidase IV/CD26 of neuropeptides involved in pancreatic insulin secretion. *FEBS Lett.* (2001) 507:327-330.
55. ZHU L, TAMVAKOPOULOS C, XIE D *et al.*: The role of dipeptidyl peptidase IV in the cleavage of glucagon family peptides: *in vivo* metabolism of pituitary adenylate cyclase activarting polypeptide-1(1-38). *J. Biol. Chem.* (2003) 278:22418-22423.
56. FILIPSSON K, REIMER MK, AHRÉN B: The neuropeptide pituitary adenylate cyclase-activating polypeptide and islet function. *Diabetes* (2001) 50:1959-1969.
57. SCHAFER H, ZHENG J, GUNDLACH F *et al.*: Pituitary adenylate-cyclase-activating polypeptide stimulates protooncogene expression and activates the AP-1 (c-Fos/c-Jun) transcription factor in ARA-2J pancreatic carcinoma cells. *Eur. J. Biochem.* (1996) 242:467-476.
58. HASHIMOTO H, KUNUGI A, ARAKAWA N *et al.*: Possible involvement of a cyclic AMP-dependent mechanism in PACAP-induced proliferation and ERK activation in astrocytes. *Biochem. Biophys. Res. Commun.* (2003) 311:337-343.
59. DIETRICH JB: Neuropeptides, antagonists and cell proliferation: bombesin as an example. *Cell Mol. Biol.* (1994) 40:731-746.
60. BHAVE SV, HOFFMAN PL: Phosphatidylinositol 3′-OH kinase and protein kinase A pathways mediate the anti-apoptotic effect of pituitary adenylyl cyclase-activating polypeptide in cultured cerebellar granule neurons: modulation by ethanol. *J. Neurochem.* (2004) 88:359-369.
61. YAMAMOTO K, HASHIMOTO H, TOMIMOTO S *et al.*: Overexrpession of PACAP in transgenic mouse pancreatic β-cells enhances insulin secretion and ameliorates streptozotocin-induced diabetes. *Diabetes* (2003) 82:1155-1162.
62. YADA T, SAKURADA M, FILIPSSON K, KIKUCHI M, AHRÉN B: Intraperitoneal PACAP administration decreases blood glucose in GK rats, and in normal and high-fat diet mice. *Ann. NY Acad. Sci.* (2000) 921:259-263.
63. MADSBAD S, SCHMITZ O, RANSTAM J, JAKOBSEN G, MATTHEWS DR: Improved glycemic control with no weight increase in patients with Type 2 diabetes after once-daily treatment with the long-acting glucagon-like peptide 1 analog Liraglutide (NN2211): A 12-week, double-blind, randomized, controlled trial. *Diabetes Care* (2004) 27:1335-1342.
64. FINEMAN MS, BICSAK TA, SHEN LZ *et al.*: Effect on glycemic control of exenatide (synthetic exendin-4) additive to existing metformin and/or sulfonylurea treatment in patients with Type 2 diabetes. *Diabetes Care* (2003) 26:2370-2377.
65. DEFRONZO R, RATNER R, HAN J, KIM D, FINEMAN M, BARON A: Effects of exenatide (synthetic exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with Type 2 diabetes. *64th Scientific Sessions of the American Diabetes Association.* Orlando, USA (2004) Late-breaking 6-LB (Abstract).
66. NAUCK MA, HOMPESCH M, FILIPCZAK R *et al.*: Liraglutide significantly improves glycemic control and reduces body weight compared with glimepiride as add-on to metformin in Type 2 diabetes. *Diabetes* (2004) 53(Suppl. 2):A83, 356-OR (Abstract).
67. HANSOTIA T, BAGGIO LL, DELMEIRE D *et al.*: Double incretin receptor knockout (DIRKO) mice reveal an essential role for the enteroinsular axis in transducing the glucoregulatory actions of DPP-IV inhibitors. *Diabetes* (2004) 53:1326-1335.
68. NAGAKURA T, YASUDA N, YAMAZAKI K, IKUTA H, TANAKA I: Enteroinsular axis of *db/db* mice and efficacy of dipeptidyl peptidase IV inhibition. *Metabolism* (2003) 52:81-86.
69. YASUDA N, INOUE T, NAGAKURA T *et al.*: Metformin causes reduction of food intake and body weight gain, and improvement of glucose intolerance in combination with dipeptidyl peptidase IV inhibitor in Zucker fa/fa rats. *J. Pharmacol. Exp. Ther.* (2004) 310(2):614-619.
70. TAKASAKI K, NAKAJIMA T, UENO K, NOMOTO Y, HIGO K: Effects of combination treatment with dipeptidyl peptidase IV inhibitor and sulfonylurea on glucose levels in rats. *J. Pharmacol.* (2004) 95:291-293.
71. BURKEY BF, LI X, BOLOGNESE L *et al.*: Combination treatment of a DPP-IV inhibitor NVP-LAF237 with pioglitazone completely normalized glucose tolerance in adult obese Zucker rats. *Diabetes* (2002) 51(Suppl. 2):A338 (Abstract).
72. WEBER A, KIM D, BECONI M *et al.*: MK-0431 is a potent, selective dipeptidyl peptidase IV inhibitor for the treatment of Type 2 diabetes. *Diabetes* (2004) 53(Suppl. 2): A151, 633-P (Abstract).
73. LI Y, HANSOTIA T, YUSTA B, RIS F, HALBAN PA, DRUCKER DJ: Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. *J. Biol. Chem.* (2003) 278:471-478.
74. BOSE AK, MOCANU MM, MENSAH KN, BRAND CL, CARR RD, YELLON DM: GLP-1 protects ischemic and reperfused myocardium via PI3 kinase and p42/p44 MAPK signalling pathways. *Diabetes* (2004) 53(Suppl. 2):A1, 2-OR (Abstract).

75. VILSBØLL T, KRARUP T, DEACON CF, MADSBAD S, HOLST JJ: Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in Type 2 diabetic patients. *Diabetes* (2001) 50:609-613.

76. MEIER JJ, NAUCK MA, KRANZ D *et al.*: Secretion, degradation, and elimination of glucagon-like peptide 1 and gastric inhibitory polypeptide in patients with chronic renal insufficiency and healthy control subjects. *Diabetes* (2004) 53:654-662.

77. HERMAN GA, ZHAO P-L, DIETRICH B *et al.*: The DP-IV inhibitor MK-0431 enhances active GLP-1 and reduces glucose following an OGTT in Type 2 diabetics. *Diabetes* (2004) 53(Suppl. 2):A82, 353-OR (Abstract).

78. LARSEN MO, ROLIN B, RIBEL U *et al.*: Valine pyrrolidide preserves intact glucose-dependent insulinotropic peptide and improves abnormal glucose tolerance in minipigs with reduced beta-cell mass. *Exp. Diabesity Res.* (2003) 4:93-105.

79. NAUCK MA, HEIMESAAT MM, ØRSKOV C, HOLST JJ, EBERT R, CREUTZFELDT W: Preserved incretin activity of glucagon-like peptide 1 (7–36 amide) but not of synthetic human gastric inhibitory polypeptide in patients with Type II diabetes mellitus. *J. Clin. Invest.* (1993) 91:301–307.

80. ELAHI D, MCALOON-DYKE M, FUKAGAWA NK *et al.*: The insulinotropic actions of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (7–37) in normal and diabetic subjects. *Regul. Pept.* (1994) 51:63–74.

81. MENEILLY GS, BRYER-ASH M, ELAHI D: The effect of glyburide on β-cell sensitivity to glucose-dependent insulinotropic polypeptide. *Diabetes Care* (1993) 16:110-114.

82. LYNN FC, PAMIR N, NG EH, MCINTOSH CH, KIEFFER TJ, PEDERSON RA: Defective glucose-dependent insulinotropic polypeptide receptor expression in diabetic fatty Zucker rats. *Diabetes* (2001) 50:1004-1011.

83. LYNN FC, THOMPSON SA, POSPISILIK JA *et al.*: A novel pathway for regulation of glucose-dependent insulinotropic polypeptide (GIP) receptor expression in beta cells. *FASEB J.* (2003) 17:91-93.

84. TANAKA S, MURAKAMI T, NONAKA N, OHNUKI T, YAMADA M, SUGITA T: Anti-arthritic effects of the novel dipeptidyl peptidase IV inhibitors TMC-2A and TSL-225. *Immunopharmacology* (1998) 40:21-26.

85. WILLIAMS YN, BABA H, HAYASHI S *et al.*: Dipeptidyl peptidase IV on activated T cells as a target molecule for therapy of rheumatoid arthritis. *Clin. Exp. Immunol.* (2003) 131:68-74.

86. JONES B, ADAMS S, MILLER GT, JESSON MI, WATANABE T, WALLNER BP: Hematopoietic stimulation by a dipeptidyl peptidase inhibitor reveals a novel regulatory mechanism and therapeutic treatment for blood cell deficiencies. *Blood* (2003) 102:1641-1648.

87. KOROM S, DE MEESTER I, STADLBAUER TH *et al.*: Inhibition of CD26/dipeptidyl peptidase IV activity *in vivo* prolongs cardiac allograft survival in rat recipients. *Transplantation* (1997) 63:1495-1500.

88. STEINBRECHER A, REINHOLD D, QUIGLEY L *et al.*: Targeting dipeptidyl peptidase IV (CD26) suppresses autoimmune encephalomyelitis and up-regulates TGF-beta 1 secretion *in vivo*. *J. Immunol.* (2001) 166:2041-2048.

89. RASMUSSEN H B, BRANNER S, WIBERG FC, WAGTMANN N: Crystal structure of human dipeptidyl peptidase IV/CD26 in complex with a substrate analog. *Nat. Struct. Biol.* (2003) 10:19-25.
- **First description of the crystal structure of human DPP IV.**

90. OEFNER C, D'ARCY A, MacSWEENEY A, PIERAU S, GARDINER R, DALE GE: High-resolution structure of human apo dipeptidyl peptidase IV/CD26 and its complex with 1-[([2-[(5-iodopyridin-2-yl)amino]-ethyl]amino)-acetyl]-2-cyano-(S)-pyrrolidine. *Acta. Crystallogr. D. Biol. Crystallogr.* (2003) 59:1206-1212.

91. THOMA R, LOFFLER B, STIHLE M, HUBER W, RUF A, HENNIG M: Structural basis of proline-specific exopeptidase activity as observed in human dipeptidyl peptidase-IV. *Structure* (2003) 1:947-959.

92. HUPE-SODMANN K, McGREGOR GP, BRIDENBAUGH R *et al.*: Characterisation of the processing by human neutral endopeptidase 24.11 of GLP-1(7-36) amide and comparison of the substrate specificity of the enzyme for other glucagon-like peptides. *Regul. Pept.* (1995) 58:149-156.

93. GEE NS, BOWES MA, BUCK P, KENNY AJ: An immunoradiometric assay for endopeptidase-24.11 shows it to be a widely distributed enzyme in pig tissues. *Biochem. J.* (1985) 228:119-126.

94. PLAMBOECK A, HOLST JJ, CARR RD, DEACON CF: Neutral endopeptidase 24.11 and dipeptidyl peptidase IV are both involved in regulating the metabolic stability of glucagon-like peptide-1 *in vivo*. *Adv. Exp. Med. Biol.* (2002) 524:303-312.

95. OEFNER C, PIERAU S, DALE GE: Ectopeptidases and Type II diabetes. *11th Protein Structure Determination in Industry*, Cambridge, UK (2003) T001 (Abstract).

96. ZHANG BB, WOODS JW, LI Z *et al.*: Chronic inhibition of dipeptidyl peptidase IV increases pancreatic beta cell mass in a murine model of Type 2 diabetes. *64th Scientific Sessions of the American Diabetes Association*. Orlando, USA (2004) Late-breaking 58-LB (Abstract).

97. STÖCKEL-MASCHEK A, MRESTANI-KLAUS C, STIEBITZ B, DEMUTH HU, NEUBERT K: Thioxo aminoacid pyrrolidides and thiazolidides: new inhibitors of proline specific peptidases. *Biochim Biophys Acta* (2000) 1479:15-31.

98. VILLHAUER EB, BRINKMAN JA, NADERI GB *et al.*: 1-[[(3-hydroxy-1-adamantyl)amino]acetyl]-2-cyano-*(S)*-pyrrolidine: a potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties. *J. Med. Chem.* (2003) 46:2774-2789.

Affiliation

Carolyn F Deacon[1†], Bo Ahrén[2] & Jens J Holst[1]
[†]Author for correspondence
[1]Department of Medical Physiology, Panum Institute, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark
Tel: +45 3532 7523; Fax +45 3532 7537;
E-mail: deacon@mfi.ku.dk
[2]Department of Medicine, University of Lund, SE-22184 Lund, Sweden

Expert Opinion

Dipeptidyl peptidase IV inhibitors as new therapeutic agents for the treatment of Type 2 diabetes

1. Introduction
2. Incretins and their metabolism as new targets in antidiabetic research
3. Dipeptidyl peptidase IV inhibitors
4. Dipeptidyl peptidase IV inhibitors as therapeutic agents for the treatment of Type 2 diabetes
5. Expert opinion

Koen Augustyns[†], Pieter Van der Veken, Kristel Senten & Achiel Haemers
[†]*Department of Medicinal Chemistry, University of Antwerp, Universiteitsplein 1, B-2610, Antwerpen, Belgium*

Type 2 diabetes is the most prevalent form of diabetes. Incretin hormones play an important role in normal and pathological blood glucose homeostasis. The role of dipeptidyl peptidase IV (DPP IV) in the inactivation of glucagon-like peptide-1 (GLP-1), one of the most important incretins, is well-established. Therefore, DPP IV inhibitors are investigated as new therapeutic agents for the treatment of Type 2 diabetes. A summary of DPP IV inhibitors reported until 1998 and a more extensive discussion of more recent inhibitors found in literature and patent applications will be provided. The therapeutic potential of several aminoacyl pyrrolidides, aminoacyl thiazolidides and aminoacyl pyrrolidine-2-nitriles will be reviewed.

Keywords: dipeptidyl peptidase (DPP) IV, DPP IV inhibitors, glucagon-like peptide-1 (GLP-1), incretin hormones, Type 2 diabetes

Expert Opin. Ther. Patents (2003) 13(4):499-510

1. Introduction

Diabetes is a chronic disease that features abnormal glucose homeostasis. About 10% of patients have Type 1 diabetes whereas 90% have Type 2 diabetes, also known as non-insulin dependent diabetes mellitus (NIDDM). Type 2 diabetes develops in middle or later life and is largely associated with obesity. Insulin resistance is an early feature of Type 2 diabetes, which is initially compensated, in part, by increased production of insulin by pancreatic β cells. Subsequently, as these cells become exhausted, the production of insulin decreases. The combined effects of insulin resistance and impaired insulin secretion reduce insulin-mediated glucose uptake and utilisation by skeletal muscle and prevent insulin-mediated suppression of hepatic glucose output. This leads to abnormally high sugar values in blood (hyperglycaemia). Chronic hyperglycaemia causes non-enzymatic glycation of proteins, osmotic effects and metabolic consequences but is in itself not sufficient to explain the chronic complications of the disease. Importantly, insulin resistance is also implicated in major diseases including atherosclerotic cardiovascular disease and dyslipidaemia (metabolic syndrome). Chronic complications such as retinopathy, nephropathy, neuropathy and atherosclerosis are the major problems for Type 2 diabetic patients suffering from poor glycaemic control.

Currently, Type 2 diabetic patients are treated by a combination of diet and exercise or with insulin and various oral pharmacological agents. These agents have several targets to reverse several aspects of the disease. Exogenous insulin, to supplement endogenous insulin supplies, is used when other treatment options fail. Sulfonylureas and glinides stimulate the residual insulin secretion. Biguanides, such as metformin and thiazolidinediones, counter insulin resistance by decreasing hepatic glucose output and increasing muscle insulin sensitivity. Thiazolidinediones exert their action by binding to the nuclear peroxisome proliferator activated receptor (PPAR)-γ. The mechanism of action of metformin is not yet completely understood. Acarbose is an α-glucosidase inhibitor that reduces the rate of intestinal

carbohydrate digestion and therefore reduces absorption. In patients where the disease is more advanced, such drugs are frequently used in combination to achieve better glycaemic control. Each of the above oral agents suffers from inadequate efficacy and a number of serious adverse effects. Insulin and sulfonylureas can cause hypoglycaemia; metformin sometimes causes lactic acidosis and acarbose is responsible for gastrointestinal disturbances. As a consequence, there continues to be a high demand for new antidiabetic agents [1,2].

2. Incretins and their metabolism as new targets in antidiabetic research

The discovery and effects of incretin hormones (incretins) are excellently described in recent reviews [3,4]. In this paper we summarise the aspects necessary to understand the development of dipeptidyl peptidase (DPP) IV inhibitors. Incretins are insulinotropic hormones of the gut that are released by nutrients and that stimulate insulin secretion in physiological concentrations in the presence of elevated blood glucose levels. It was observed that glucose administered orally gave rise to much higher insulin levels than intravenously administered glucose, in spite of similar or even higher plasma glucose levels obtained with the latter. This incretin effect is defined as the ratio between the integrated insulin response to an oral glucose load and an isoglycaemic intravenous glucose infusion. The incretins form the hormonal component of the entero-insular axis. This term describes the connection between the gut and the pancreatic islets and comprises all stimuli coming from the small intestine and influencing the release of different islet hormones, including hormonal, nervous and direct substrate stimulation. It has been shown that the incretin function is greatly impaired in Type 2 diabetes [5].

Today, there is general agreement that the two most important incretin hormones are glucose-dependent insulinotropic polypeptide (GIP, formerly known as gastric inhibitory polypeptide) and glucagon-like peptide-1 (GLP-1). GLP-1 refers to the biologically active form of glucagon-like peptide-1, either amidated (GLP-1 [7-36] amide) or with a C-terminal glycine (GLP-1 [7-37]). Both GIP and GLP-1 are potent insulinotropic hormones and both are released by oral glucose as well as ingestion of mixed meals. GIP is a peptide of 42 amino acids belonging to the glucagon-secretin family. It is secreted from specific endocrine cells, the so-called K cells, which exhibit the highest density in the upper small intestine (duodenum and jejunum). Secretion is stimulated by absorbable carbohydrates and lipids. GLP-1 is a peptide of 30 amino acids belonging to the same family as GIP. It is secreted from L cells, which exhibit the highest density in the lower small intestine (ileum) and the colon. GLP-1 is one of the most potent insulin-releasing substances known, its potency actually exceeding that of GIP [3,4].

As mentioned above, interference with the incretin action of GLP-1 and GIP results in glucose intolerance in Type 2 diabetic patients. It seems that the major components of incretin defect in Type 2 diabetes are a defective secretion of GLP-1 and a defective insulinotropic activity of GIP. On the other hand, the insulinotropic activity of GLP-1 and the secretion of GIP are more or less preserved. Indeed, it was shown that supraphysiological (pharmacological) concentrations of GLP-1 can normalise elevated blood glucose levels in Type 2 diabetic patients, whereas supraphysiological concentrations of GIP have no effect [6]. This suggests a therapeutic use of GLP-1 for the treatment of Type 2 diabetes.

Furthermore, GLP-1 has a number of effects that are highly desirable in the context of treatment of Type 2 diabetes (reviewed in [3]):

- GLP-1 is released upon absorption of nutrients from the lower gut, followed by a glucose-dependent increase in the secretion of insulin. The glucose dependency of secretion and insulinotropic effect of GLP-1 has been interpreted as a safeguard against hypoglycaemia.
- GLP-1 stimulates all steps of insulin biosynthesis and insulin gene transcription.
- GLP-1 upregulates the genes involved in insulin secretion.
- GLP-1 stimulates β cell proliferation and enhances the differentiation of new β cells in rodents.
- GLP-1 strongly inhibits glucagon secretion.
- GLP-1 inhibits gastrointestinal secretion, motility and gastric emptying.
- GLP-1 inhibits appetite and food intake.

Near normalisation of diurnal plasma glucose concentrations were obtained during continuous intravenous infusion of GLP-1 in Type 2 diabetic patients [7]. However, it turned out that simple subcutaneous injections of GLP-1 are ineffective [8]. The reason for this is that GLP-1 is metabolised extremely rapidly by the ubiquitous enzyme DPP IV (EC 3.4.14.5). DPP IV cleaves a dipeptide from GLP-1, which is thereby inactivated. In fact, the metabolite (GLP-1[9-36]) may act as a GLP-1 receptor antagonist [9].

DPP IV is a serine protease cleaving off Xaa–Pro or Xaa–Ala dipeptides from the N terminus of peptides. DPP IV is identical to the T cell differentiation antigen CD26 and is considered to be involved in T cell costimulation [10]. It is a transmembrane protein but is also found in human serum in a soluble form lacking the transmembrane region. Intestinal and renal DPP IV are involved in digestion of proline-containing peptides. More importantly, DPP IV cleavage causes an alteration in receptor interaction of several regulatory peptides with N-terminal Xaa–Pro or Xaa–Ala dipeptides or even causes their complete inactivation. It plays an important role in degradation of neuropeptides (substance P, endomorphin 2), members of the pancreatic polypeptide family (neuropeptide Y, peptide YY), members of the glucagon-secretin family (GLP-1, GLP-2, GIP, growth hormone-releasing factor (GRF), peptide histidine methionine (PHM)) and several chemokines [10-13]. However, most studies reporting cleavage of synthetic peptide substrates are under *in vitro* conditions, making predictions of a physiological role for

DPP IV in the control of the biological activity of these substrates speculative. The therapeutic potential of DPP IV inhibitors regarding these substrates has been reviewed [13,14].

The inactivation of GLP-1 and GIP by DPP IV, both *in vitro* and *in vivo*, has been clearly established [15,16]. It has been shown that endogenous GLP-1 is already metabolised before it leaves the gut. Whereas all of the peptide stored in the L cells is intact, two-thirds of that leaving the gut is degraded by DPP IV, present in the endothelium of the capillaries surrounding the gut [17]. Also, the majority of exogenous GLP-1, whether administered intravenously or subcutaneously, is present in the circulation as the truncated, inactive metabolite [18]. Thus, it is clear that GLP-1 itself cannot be used for the treatment of Type 2 diabetes.

To solve this problem, a number of different strategies have been explored:

- Development of small molecule agonists for the GLP-1 receptor. Until now, this approach has not been successful.
- Development of DPP IV-resistant analogues (e.g., replacement of Ala at the penultimate position) [19,20].
- Alternative routes of application, such as continuous subcutaneous infusion of GLP-1 [19].
- Inhibition of DPP IV [21].

The use of DPP IV inhibitors for the treatment of Type 2 diabetes has been investigated extensively. As discussed below, DPP IV inhibitors improve glucose tolerance during shor-term studies in normal and diabetic rodents, pigs, monkeys, healthy volunteers and Type 2 diabetic patients. Further evidence for the importance of DPP IV inhibition comes from two different animal models. Targeted inactivation of the CD26 gene in mice (CD26(-/-) mice) yielded apparently healthy mice that have normal blood glucose levels in the fasted state but reduced glycaemic excursion after a glucose challenge. Levels of glucose-stimulated circulating insulin and intact GLP-1 are increased in these animals. A DPP IV inhibitor improved glucose tolerance in wild type but not in CD26(-/-) mice [22]. Comparable results were obtained in DPP IV-deficient rats [23]. The same DPP IV-deficient rats also showed an improvement in insulin resistance induced by a high fat diet [24].

Selectivity is an issue that needs further investigation in order to determine the value of DPP IV inhibitors in the treatment of Type 2 diabetes. DPP IV itself has multiple substrates, many of which are probably not yet known. As mentioned before, apart from the incretins GLP-1 and GIP, several neuropeptides, peptide hormones and chemokines are substrates for DPP IV. The importance of DPP IV inhibition in these other systems is not known but a major consideration would be whether DPP IV is the primary inactivating pathway for these substrates *in vivo*. Another potential problem arises from the function of CD26 (DPP IV) in the immune system. Furthermore, the validation of the therapeutic potential of DPP IV inhibitors *in vivo* is complicated by a multiplicity of enzymes reported to exhibit DPP IV-like activity, including: DPP II or quiescent cell proline dipeptidase (QPP), DPP IVβ, DPP 8, DPP 9 and fibroblast-activation protein (FAP) [25]. The physiological role of these proteases in the metabolism of incretins or other substrates has not yet been investigated. Also, the selectivity of the reported DPP IV inhibitors with respect to these enzymes is an important issue when considering possible long-term unwanted effects.

3. Dipeptidyl peptidase IV inhibitors

DPP IV is typically inhibited by dipeptide analogues containing an electrophilic group at the P_1 amino acid, replacing the normally cleaved amide bond. This electrophile can interact with the hydroxyl of the catalytic serine in the active site but it is also responsible for stability problems due to reaction with the free amino group of the P_2 amino acid. For a discussion on DPP IV inhibitors reported until 1998 the reader is referred to a review by Augustyns *et al.* [13]. In the present paper, only some representative examples from the most important inhibitor classes will be mentioned (**Figure 1**). In the next chapter the more recent additions from literature and patent applications will be reviewed.

3.1 Summary of dipeptidyl peptidase IV inhibitors reported until 1998

The simplest inhibitors of DPP IV are product-like compounds lacking the carbonyl function of the proline residue, such as aminoacyl pyrrolidides and thiazolidides. These are competitive, reversible inhibitors with IC_{50} values in the low micromolar range. The most potent compound of this series was *N*-isoleucylthiazolidide (compound 1; **Figure 1**) [26]. This compound is currently being developed by Probiodrug under the name P32/98. Changing the size of the 5-membered ring, introduction of a substituent or replacement with acyclic amines decreased potency. Only a small substituent such as fluorine is allowed [27]. To establish an optimal N-terminal residue, a series of aminoacyl pyrrolidides were prepared, showing that lipophilic amino acids gave more potent compounds. In particular, β branched α-amino acid derivatives such as cyclohexylglycylpyrrolidide showed high potency (compound 2) [28].

A series of pyrrolidine-2-nitriles were developed with, on average, a 1000-fold increase in potency compared to the pyrrolidides [28,29]. The structure–activity relationship for the N-terminal residue developed in the pyrrolidide series correlated well for the dipeptide nitriles, with the cyclopentylglycine derivative 3 as a very potent compound. This compound has a K_i value of 1.1 nM and an excellent chemical stability at pH = 7.4 ($t_{½}$ = 48 h). In this series, Ferring is currently testing nitrile 4 (FE 999011, K_i = 3.8 nM) [101] as a DPP IV inhibitor for the treatment of Type 2 diabetes [30]. Enhancement of lipophilicity was also achieved by the introduction of long alkyl chains in the side chain of the P_2 amino acid (compound 5, K_i = 0.5 nM) [101]. In analogy

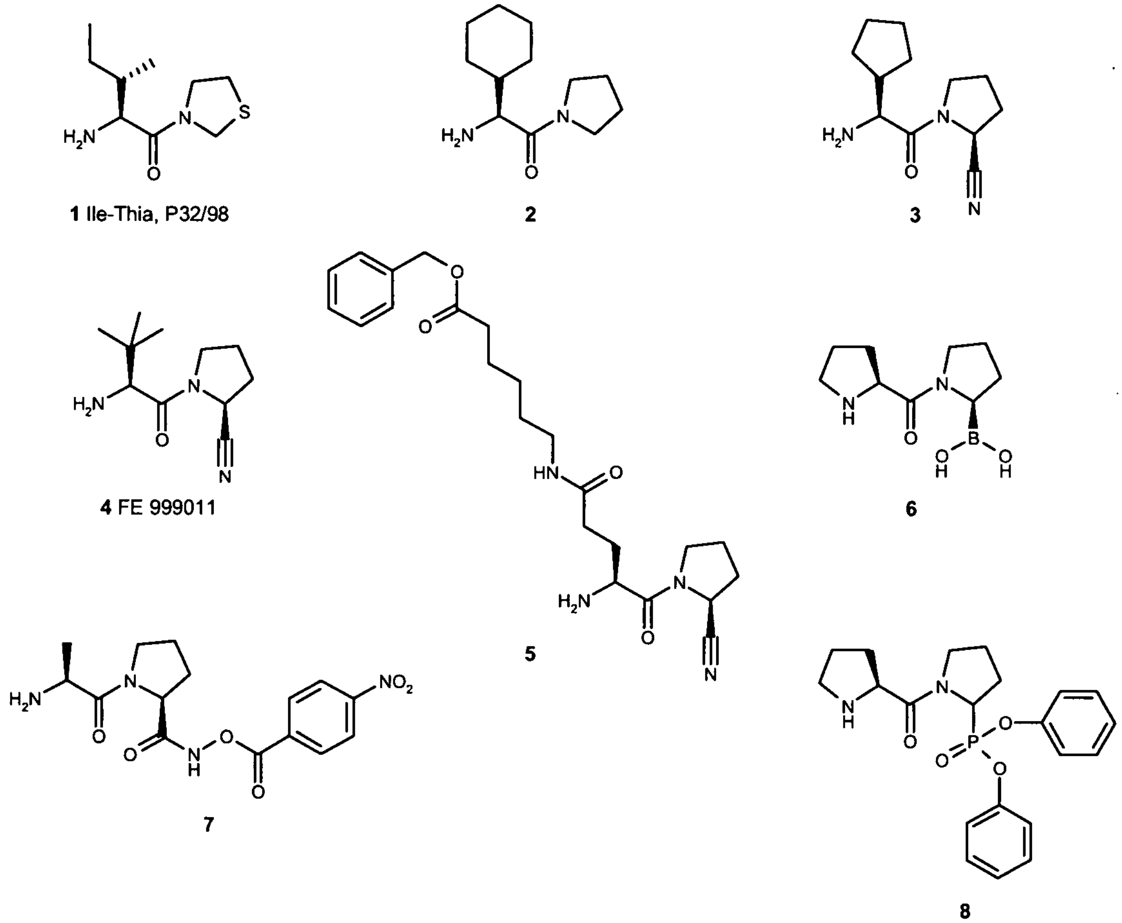


Figure 1. Summary of dipeptidyl peptidase IV inhibitors reported until 1998.

with the pyrrolidides, replacement of the pyrrolidine-2-nitrile with thiazolidine-4-nitrile enhanced potency [31].

L-Pro-L-boroPro (compound 6) is probably the most potent DPP IV inhibitor with a K_i value of 16 pM [32]. This series of compounds are reversible transition-state analogues with slow-tight binding kinetics [33]. Unfortunately, they have a very short half-life at neutral pH, caused by cyclisation of the terminal aminofunction with the boronic acid, forming a cyclic, inactive species containing a B–N bond.

N-Peptidyl-*O*-(4-nitrobenzoyl)hydroxylamines, such as compound 7, are enzyme-activated irreversible inhibitors of serine proteases [34]. After attack of the active site serine, the inhibitor forms a chemically reactive intermediate, capable of reacting with a nucleophile at or near the active site.

Dipeptide-derived diphenyl phosphonate esters form another class of potent, irreversible DPP IV inhibitors [35], leading to a phosphorylated serine at the active site. In a study on the role of the P_2 amino acid in dipeptide diphenyl phosphonates [36], it was shown that proline in this position gives one of the most potent inhibitors (compound 8). A major advantage of compound 8 was its improved stability in human citrated plasma ($t_{½}$ = 5 h) compared to the other dipeptide derivatives. Intravenous injection of a single dose of compound 8 (1, 5 or 10 mg) in rabbits reduces plasma DPP IV activity by > 80% and it takes > 20 days for complete recovery. Not only plasma DPP IV was inhibited but also DPP IV in circulating lymphocytes and peripheral tissues [37].

3.2 Dipeptidyl peptidase IV inhibitors reported between 1999 and 2002

The latest developments in the above mentioned inhibitor classes, as well as totally new concepts are discussed in this chapter.

Probiodrug is developing pro-drugs of their DPP IV inhibitor isoleucylthiazolidide (1, Ile-Thia, P32/98). One example is Gly-Pro-Ile-Thia (compound 9; Figure 2) [102]. This peptide derivative is a substrate for DPP IV that cleaves after proline and thereby releases the inhibitor Ile-Thia. It is described that this pro-drug has a 30% increased effect on glucose tolerance in Wistar rats compared to the parent compound. This is not due to an increase in bioavailability, since the parent compound is 100% available. The authors claim that through

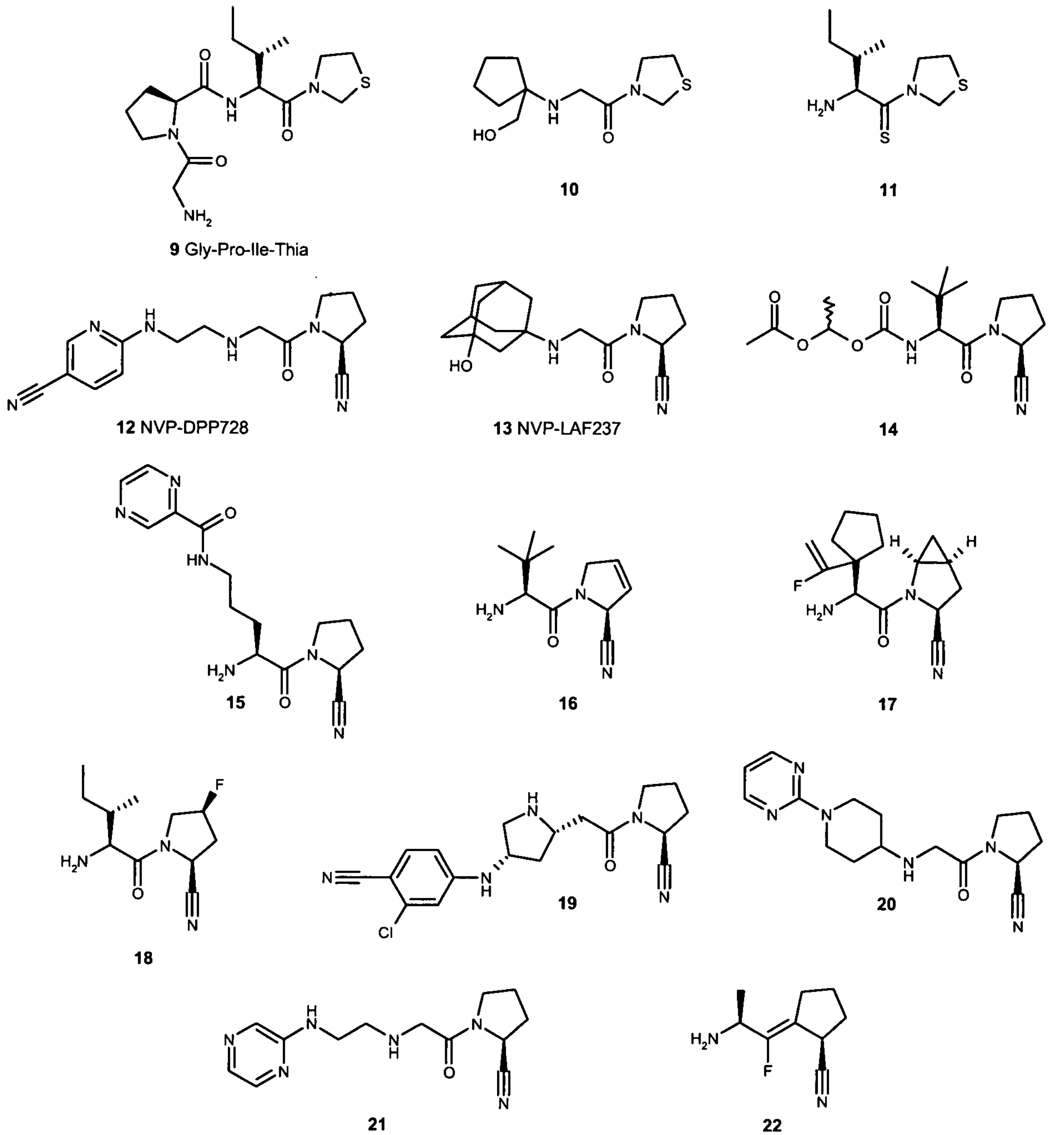


Figure 2. Aminoacyl thiazolidide and pyrrolidine-2-nitrile dipeptidyl peptidase IV inhibitors.

cleavage of the pro-drug by DPP IV, DPP IV is inhibited gradually, ultimately resulting in termination of the activation of the pro-drug. The uncleaved pro-drug will then act as a depot. Due to this effect it would be possible to release the inhibitor in an amount adjusted to the level of DPP IV in individual patients and to the level of DPP IV in different tissues and at different time points.

Novartis synthesised *N*-(substituted glycyl)thiazolidides [103]. These are peptoid like molecules, in which the side chain has moved from the α-carbon to the terminal nitrogen. The most potent compound from this series is **10**, with an IC_{50} value of 5.7 µM on human plasma DPP IV. However, no comparison with reference compounds was made. The same peptoid principle was also applied to the pyrrolidine-2-nitriles (*vide infra*).

Thioamide analogues of the above mentioned aminoacyl pyrrolidides and thiazolidides were reported [38]. However, thioxylation led to a slight decrease in inhibitory potency.

DPP IV is only inhibited by the *Z* isomer of the thioamide bond. Ile-ψ[CS-N]-Thia (compound 11) has a K_i value of 0.203 μM on DPP IV from pig kidney, compared to a K_i value of 0.126 μM for Ile-Thia (1). In the same paper, the authors describe that thioxylation increases the inhibitory potency towards DPP II, thereby reducing the selectivity of these compounds for DPP IV. In general, aminoacyl pyrrolidides and thiazolidides have a low selectivity index when DPP IV inhibition is compared with DPP II inhibition [38,39]. For example, Ile-Thia (1, P32/98) has a selectivity index of 65 in favour of DPP IV. The selectivity index here is defined as the K_i value for DPP II divided by the K_i value for DPP IV.

Novartis also applied the above mentioned peptoid principle to a large series of pyrrolidine-2-nitriles [104] and thiazolidine-4-nitriles [105]. As described above, the thiazolidine-4-nitriles are more potent. A very active pyrrolidine-2-nitrile in this series is NVP-DPP728 (compound 12) with a K_i value of 11 nM on human plasma DPP IV [40]. The potency of NVP-DPP728 is strongly dependent upon the presence and chirality of the P_1 nitrile. By alteration of the orientation (L to D) of the nitrile-pyrrolidine bond, ~ 500-fold loss of potency was observed. By removal of the nitrile substituent (hydrogen replacement), a 1000-fold loss of potency resulted. Similarly, placement of a more bulky amide substituent in place of the nitrile resulted in a 30,000-fold loss of potency. Kinetic experiments have established that NVP-DPP728 derives its potency through a slow-binding inhibition mechanism. Formation of the high-affinity complex may be either due to a dipole–hydrogen bond interaction (interaction of the nitrile dipole with the negatively charged active site serine and a hydrogen bond donor) or due to a transient imidate formation. Under the assay conditions (pH = 7.4) the P_2 site amine can nucleophilically attack the carbon of the pyrrolidine-nitrile to form an inactive cyclic amidine. This intramolecular cyclisation was slow with a $t_{½}$ of ~ 72 h. Also, compound 12 is highly selective for DPP IV (IC_{50} for human plasma DPP IV = 7 nM) over closely related peptidases, postproline cleaving enzyme (PPCE, IC_{50} = 190,000 nM) and DPP II (IC_{50} = 110,000 nM). In addition, the *in vitro* specificity was profiled in > 100 receptor and enzyme assays and no significant binding was observed (10 μM) [41].

A further study in this direction by Novartis afforded the 3-hydroxy-adamant-1-yl derivative 13 (NVP-LAF237) [106]. Compound 13 is slightly more potent than compound 12 (IC_{50} for human plasma DPP IV = 2.7 nM and 7 nM, respectively). It is also highly specific, showing > 75,000-fold selectivity for DPP IV relative to other enzymes profiled (prolyl oligopeptidase, DPP II, trypsin and aminopeptidase P) [42].

Ferring synthesised pro-drugs of their pyrrolidine-2-nitriles in order to prevent cyclisation and hence increase chemical stability [107]. An example of this approach is compound 14, an acetoxyethoxycarbonyl pro-drug of compound 4 (FE 999011). These pro-drugs show no significant inhibitory activity on DPP IV up to 10 μM, indicating that the pro-drugs are ≥ 1000 times less potent than the parent compounds. Hence it can be assumed that any *in vivo* activity seen is due to bioconversion into the parent inhibitors. Ferring also went further along the line of the side chain substituted P_2 analogues, exemplified by compound 15 [108]. It is stated that all 450 examples are competitive inhibitors with K_i values < 300 nM but no specific biological data are reported. Furthermore, it is reported that these DPP IV inhibitors are effective in reducing hyperglycaemia in Zucker obese rats.

In patent literature, several analogues of the pyrrolidine-2-nitrile series are described by different companies but either no biological data are described or the added value is unclear (compounds 16 – 21) [109-114].

In order to increase the chemical stability of aminoacyl pyrrolidine-2-nitriles, the amide bond has been replaced with a (*Z*)-fluoroolefin (compound 22) [43]. The (*Z*)-fluoroolefin is a peptidomimetic of a trans amide bond and prevents isomerisation to a cis bond, necessary for the cyclisation reaction responsible for instability. Compound 22 was isolated as two pairs of diastereomers. Unexpectedly, both pairs had similar inhibitory potency on DPP IV (K_i value of 7.69 and 6.03 μM). This is in contrast with the absolute requirement of an L,L-stereochemistry for dipeptide inhibitors with Pro at P_1. Unfortunately, the authors did not compare the fluoroolefin 22 with the corresponding Ala-Pyrr-2-CN under their assay conditions. A K_i of 0.2 μM has been reported for the latter compound [29], that seems to indicate that replacement of amide with fluoroolefin decreases potency. Likewise, it is stated that the fluoroolefin 22 is very stable but no comparison is made with the parent amide.

It is well known that boroPro inhibitors such as compound 6 cyclise to an inactive compound with a B–N bond. The linear chain (active inhibitor) is favoured at low pH, whereas the inactive cyclic compound is favoured at high pH. However, cyclic analogues such as compound 23 (**Figure 3**) can also be administered orally as pro-drugs. The acidic conditions in the stomach are sufficient to regenerate the linear chain and regain its inhibitory activity [115].

Similar to the (*Z*)-fluoroolefin nitrile 22, a (*Z*)-fluoroolefin *N*-peptidyl-*O*-(4-nitrobenzoyl)hydroxylamine, compound 24, was isolated as two pairs of diastereomers [44]. In this example, it was proven that introduction of a (*Z*)-fluoroolefin as a peptidomimetic of a trans amide bond, enhances both potency and chemical stability. The pair of diastereomers containing *S*, *R* (mimic of an L,L dipeptide) and *R*, *S* exhibited an inhibitory activity superior to 7 (K_i = 0.19 μM and 30 μM, respectively). The other pair of diastereomers containing *S*, *S* and *R*, *R* was less potent (K_i = 14.4 μM) than the first pair of diastereomers but still more potent than the parent amide. Chemical stability in buffer (pH = 7.6) of the first pair of diastereomers compared to 7 was also enhanced ($t_{½}$ = 103 h versus 8.8 h).

With the diphenyl phosphonate 8 as the lead compound, a series was synthesised with different substituents on the aryl rings. A good correlation was found between the electronic

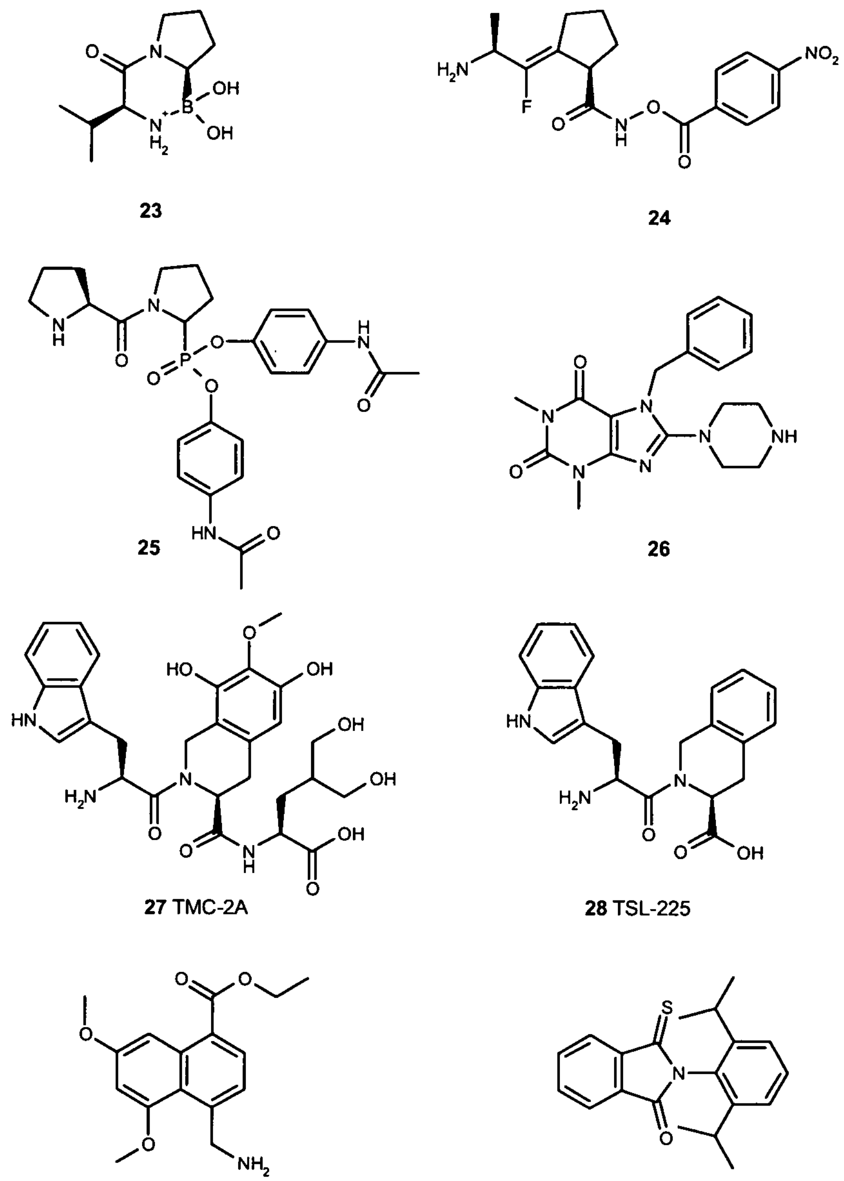


Figure 3. Other dipeptidyl peptidase IV inhibitors.

properties of the substituent and the inhibitory activity and stability in plasma. This means that electron-withdrawing substituents increase potency but unfortunately also decrease stability. The most striking divergence of this correlation was the high potency combined with a high stability in plasma of the 4-acetylamino substituted derivative 25 (IC_{50} = 0.4 µM, $t_{½}$ = 320 min) [45,116]. This compound is highly specific for DPP IV and shows low cytotoxicity in human peripheral blood mononuclear cells.

So far, the mentioned inhibitors are structurally derived from the dipeptide obtained after DPP IV cleavage. At Novo Nordisk a completely novel class of inhibitor was discovered, structurally unrelated to any DPP IV inhibitor known so far. This class is exemplified by 7-benzyl-1,3dimethyl-8-(1-piperazinyl)xanthine (compound 26) [117]. It is claimed that these compounds are furthermore potent, competitive and stable, thus offering a solution to the instability problems often associated with the previously known DPP IV inhibitors [46]. Compound 26 has favourable pharmacokinetic properties in rats and is active in an oral glucose tolerance test in the Zucker obese rat model.

Novel DPP IV inhibitors were discovered from the fermentation broth of *Aspergillus orizae* A374. TMC-2A (compound 27) inhibited DPP IV in a non-competitive manner (K_i = 5.3 µM) with high selectivity. An approach using combinatorial synthesis identified TSL-225 (compound 28) as

the critical core structure responsible for the inhibitory activity (K_i = 3.6 μM). *In vivo* evaluation revealed that both compounds had antiarthritic effects, although their mechanism of action remains to be clarified [47,48].

Compound 29 was identified as the most potent DPP IV inhibitor (IC_{50} = 0.32 μM) in a series of 1-aminomethylisoquinoline-4-carboxylates [49]. Within this series, a primary amine is required for inhibitory activity and the 6,8-dimethoxy substitution is optimal.

Compound 30 is reported as a DPP IV inhibitor (IC_{50} = 57.8 μM) with selectivity towards aminopeptidase N (IC_{50} > 309 μM), but with cytotoxic properties (IC_{50} = 187 μM). The authors use an intact-cell assay and report that under their conditions, compound 30 is more potent than Pro-boroPro (compound 6; IC_{50} = 88.2 μM).

4. Dipeptidyl peptidase IV inhibitors as therapeutic agents for the treatment of Type 2 diabetes

Currently, several DPP IV inhibitors are in preclinical and clinical studies for the treatment of Type 2 diabetes. In the literature, data on the following compounds were found: *N*-isoleucylthiazolidide (1, Ile-Thia, P32/98), *N*-valylpyrrolidide (Val-Pyrr), FE 999011 (4), NVP-DAP728 (12) and NVP-LAF237 (13).

Inhibition of circulating DPP IV [50] with orally administered Ile-Thia (1) enhanced insulin secretion and improved glucose tolerance in response to an oral glucose challenge in lean and obese Zucker rats. The enhanced incretin response was greater in obese than in lean animals, with a more profound improvement in glucose tolerance [51]. This encouraging result on the acute effects of treatment with compound 1 was followed by a study on the long-term effects [52]. Vancouver diabetic fatty (VDF) rats were treated for 3 months with compound 1 (10 mg/kg, orally, twice daily). VDF rats are a substrain of the fatty (*fa/fa*) Zucker rat, which display abnormalities characteristic of Type 2 diabetes. Oral glucose tolerance tests revealed a sustained improvement in glucose tolerance in the animals after 3 months treatment. The tests were performed after drug washout indicating that the observation was a chronic effect, rather than an acute effect. Concomitant insulin determinations showed an increased early-phase insulin response in the treated group (43% increase). Furthermore, improvements in β cell glucose responsiveness and peripheral insulin sensitivity were novel observations that provide further support for the use of DPP IV inhibitors in the treatment of Type 2 diabetes. A small clinical study showed that an oral dose of compound 1 (60 mg) improved postprandial blood glucose tolerance in patients with Type 2 diabetes. Compound 1 is currently undergoing Phase II clinical trials for the treatment of Type 2 diabetes [53].

Probiodrug is also developing pro-drugs of their DPP IV inhibitor isoleucylthiazolidide (1, Ile-Thia, P32/98). One example is Gly-Pro-Ile-Thia (9) [102]. It is described that this pro-drug has a 30% increased effect on glucose tolerance in Wistar rats compared to the parent compound.

Another inhibitor of this class (Val-pyrrolidide, Val-Pyrr) reduces the degradation of GLP-1, thereby potentiating its insulinotropic effect in the anaesthetised pig [21]. More recently, it was observed that Val-Pyrr similarly potentiates the insulinotropic effect of another incretin hormone, GIP [54]. This inhibitor also improved glucose tolerance and insulin secretion in high fat-fed glucose-intolerant mice [55].

Oral administration of the pyrrolidine-2-nitrile (FE 999011, compound 4) to Zucker fatty rats produced an immediate suppression of plasma DPP IV activity. In the same study, the effect of nitrile 12 (NVP-DPP728) was also investigated [30]. Maximal inhibition of DPP IV activity was obtained 30 min after oral administration of NVP-DPP728 (10 mg/kg) and 1 h after oral administration of FE 999011 (10 mg /kg). NVP-DPP728 significantly reduced DPP IV activity for 6 h with return to control values after 12 h. FE 999011 has a longer duration of action with a significant DPP IV inhibition for 12 h and return to control values after 24 h. This means that twice-a-day oral administration continuously inhibits DPP IV activity. In the same rats, FE 999011 dose-dependently attenuated glucose excursion during an oral glucose tolerance test and increased GLP-1[7-36] release in response to intraduodenal glucose. Administration of FE 999011 twice-a-day for 7 days improved glucose tolerance and insulin sensitivity even in the absence of inhibitor given at the time of the glucose load. Encouraged by these results, FE 999011 was tested in Zucker diabetic fatty (ZDF) rats. This is a model for Type II diabetes since these rats become diabetic at 8 weeks if fed a diet containing 6.5% fat. Prevention of the diabetic situation was possible only with the twice-a-day administration, suggesting that continuous inhibition of DPP IV is required for optimal efficacy. The onset of hyperglycaemia was delayed by 21 days. In addition, food and water intake were stabilised to prediabetic levels and hypertriglyceridaemia was reduced, while preventing the rise in circulating free fatty acids. Also, basal plasma levels of GLP-1 were increased and pancreatic gene expression for the GLP-1 receptor was upregulated. All these data suggest that FE 999011 (4) could be of clinical value to delay the progression from impaired glucose tolerance to Type 2 diabetes.

A pro-drug of this compound (14) was also found to be orally-active during an oral glucose tolerance test in Zucker fatty rats. The pro-drug was effective at reducing hyperglycaemia but was not as effective as the parent compound at the early time points. This is a result of the need for metabolic conversion of the circulating pro-drug to the parent compound [107].

The nitrile of Novartis (12, NVP-DPP728) has also been extensively investigated. Acute inhibition of DPP IV in *fa/fa* rats with an oral dose of compound 12 (10 μmol/kg) improved insulin secretion and glucose tolerance [56]. Similar short-term effects of NVP-DPP728 were observed in cynomologus monkeys [41] and humans [57]. DPP IV inhibition

with NVP-DPP728 prevented N-terminal degradation of endogenous incretins in dogs, resulting in increased plasma concentrations of intact, biologically active GIP and GLP-1. However, total incretin secretion was reduced by DPP IV inhibition, suggesting the possibility of a feedback mechanism [58]. A long-term study in mice shows an improvement in glucose tolerance and islet function [59]. This inhibitor improved glucose tolerance in wild type rats but not in DPP IV-deficient rats. These results indicate that the amelioration of glucose tolerance by NVP-DPP728 in the wild type rats was directly due to inhibition of plasma DPP IV activity [60,61].

Pharmacokinetic evaluation of NVP-DPP728 in rats and monkeys shows a high absolute bioavailability. In humans, a 100 mg oral dose provided a $t_{1/2}$ of 0.85 h, a > 80% inhibition of plasma DPP IV activity for ~ 4 h and a significant increase in active GLP-1 levels [41]. This compound was selected for a 4 week study in a clinical setting for Type 2 diabetes [57].

The short duration of action of NVP-DPP728 (12) has resulted in the need for frequent (with-meal) administration, limiting the useful duration of GLP-1 potentiation to the immediate prandial period [30,55]. NVP-LAF237 (13) has been identified, as an outcome of a search for DPP IV inhibitors, with the potential for once-daily administration. Oral administration of NVP-LAF237 (10 µmol/kg) to *fa/fa* rats results in a fast DPP IV inhibition and increased levels of intact GLP-1. Relative to Val-Pyrr and NVP-DPP728, NVP-LAF237 displayed a markedly increased duration of action in normal Sprague–Dawley rats, evidenced by increased potency 4 h postdose (ED_{50} values of 19, 14 and 1 µmol/kg). A single 1 µmol/kg dose of NVP-LAF237 inhibited plasma DPP IV activity by > 50% for ~ 10 h in Cynomologus monkeys [42]. Because of this longer duration of action, it seems that Novartis changed their development of DPP IV inhibitors from NVP-DPP728 (12) to NVP-LAF237 (13).

5. Expert opinion

In conclusion, we can state that DPP IV inhibitors have proven their therapeutic potential during short-term studies in normal and diabetic rodents, pigs, monkeys, healthy volunteers and Type 2 diabetic patients. Longer studies in rodents not only indicate an improvement in glucose tolerance but also an amelioration of other diabetic complications. It was suggested that DPP IV inhibitors could be of clinical value to delay the progression from impaired glucose tolerance to Type 2 diabetes [30]. In this same report it was also described that a continuous inhibition of DPP IV at a certain level is required to obtain an optimal effect. In this respect, the development of potent and long-acting DPP IV inhibitors will be required. A good example is the longer duration of action of the Novartis compound NVP-LAF237 (13) compared to NVP-DPP728 (12). However, long-term studies are absolutely necessary to assess the value of DPP IV inhibitors in a chronic disease such as Type 2 diabetes. Since actions of GLP-1 are glucose-dependent, GLP-1 metabolism represents an ideal target for stimulating insulin secretion without causing hypoglycaemia. Therefore, DPP IV inhibitors for the treatment of Type 2 diabetes could have an advantage over exogenous insulin and sulfonylureas that frequently cause hypoglycaemia.

Since Type 2 diabetes is a chronic disease, careful investigation of unwanted effects due to selectivity problems will become an important issue. As described above, DPP IV might be involved in the degradation of several neuropeptides, peptide hormones and chemokines. Another potential problem arises from the function of CD26 (DPP IV) in the immune system. Furthermore, the validation of the therapeutic potential of DPP IV inhibitors *in vivo* is complicated by a multiplicity of enzymes reported to exhibit DPP IV-like activity, including: DPP II or QPP, DPP IVβ, DPP 8, DPP 9 and FAP. The physiological role of these proteases in the metabolism of incretins or other substrates has not yet been investigated but the selectivity of the reported DPP IV inhibitors with respect to these enzymes might be an important issue when considering possible long-term unwanted effects. In general, aminoacyl pyrrolidides and thiazolidides have a low selectivity index when DPP IV inhibition is compared with DPP II inhibition [39]. For example, Ile-Thia (1, P32/98) has a selectivity index of 65 in favour of DPP IV [38]. Val-Pyrr is slightly more selective with a selectivity index of 102 [38] or 56 [39] in favour of DPP IV. However, administration of these compounds in diabetic animal models does not seem to cause any signs of unwanted effects. Furthermore, CD26 knockout mice (CD26(-/-) mice) and DPP IV-deficient rats are fertile and healthy. The pyrrolidine-2-nitrile inhibitors (4, 12, 13) are more favourable when considering potency and selectivity. A disadvantage of this class of compounds compared to the aminoacyl pyrrolidides and thiazolidides might be the more difficult synthesis and the lower chemical stability of the nitrile function. At this moment, there are not sufficient data to evaluate the value of the other reported DPP IV inhibitors.

The recent publication of the crystal structure of human DPP IV in complex with Val-pyrrolidide will greatly enhance the development of new DPP IV inhibitors [62]. Computer-assisted rational design of inhibitors and virtual screening will most certainly result in new classes of inhibitors that have a non-peptidic nature. Also, an explanation of the physiological functions of the different domains of DPP IV will be facilitated.

Acknowledgement

This work received support from The Fund for Scientific Research – Flanders (Belgium) (FWO) and the Special Fund for Research – University of Antwerp (BOF UA). P Van der Veken is a fellow of the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT).

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. BAILEY CJ: Potential new treatments for Type 2 diabetes. *Trends In Pharmacol. Sci.* (2000) 21:259-265.
2. ZHANG BB, MOLLER DE: New approaches in the treatment of Type 2 diabetes. *Curr. Opin. Chem. Biol.* (2000) 4:461-467.
3. HOLST JJ, ORSKOV C: Incretin hormones – an update. *Scand. J. Clin. Lab. Invest.* (2001) 61(Suppl.234):75-86.
   • **A review describing the effects and potential therapeutical applications of incretin hormones.**
4. CREUTZFELDT W: The entero-insular axis in Type 2 diabetes – incretins as therapeutic agents. *Exp. Clin. Endocrinol. Diabetes* (2001) 109(Suppl.2):S288-S303.
   •• **An excellent review on the actions of incretin hormones as well as on the historical research leading to their discovery.**
5. NAUCK MA, STOCKMANN F, EBERT R, CREUTZFELDT W: Reduced incretin effect in Type 2 (non-insulin-dependent) diabetes. *Diabetologia* (1986) 29:46-52.
6. NAUCK MA, HEIMESAAT MM, ÖRSKOV C, HOLST JJ, EBERT R, CREUTZFELDT W: Preserved incretin activity of GLP-1 [7-36 amide] but not of synthetic human GIP in patients with Type-2-diabetes mellitus. *J. Clin. Invest.* (1993) 91:301-307.
7. RACHMAN J, BARROW BA, LEVY JC, TURNER RC: Near-normalisation of diurnal glucose concentrations by continuous administration of glucagon-like peptide 1 (GLP-1) in subjects with NIDDM. *Diabetologia* (1997) 40:205-211.
8. NAUCK MA, WOLLSCHLAGER D, WERNER J, HOLST JJ, ORSKOV C, CREUTZFELDT W: Effects of subcutaneous glucagon-like peptide 1 (GLP-1 [7-36 amide]) in patients with NIDDM. *Diabetologia* (1996) 39:1546-1553.
9. KNUDSEN LB, PRIDAL L: Glucagon-like peptide-1-[9-36] amide is a major metabolite of glucagon-like peptide-1-[7-36] amide after *in vivo* administration to dogs, and it acts as an antagonist on the pancreatic receptor. *Eur. J. Pharmacol.* (1996) 318:429-435.
10. DE MEESTER I, KOROM S, VAN DAMME J, SCHARPE S: CD26, let it cut or cut it down. *Immunol. Today* (1999) 20:367-375.
11. DE MEESTER I, DURINX C, BAL G *et al.*: Natural substrates of dipeptidyl peptidase IV. *Adv. Exp. Med. Biol.* (2000) 477:67-87.
12. MENTLEIN R: Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. *Regul. Pept.* (1999) 85:9-24.
    •• **An excellent review describing the involvement of DPP IV in the metabolism of regulatory peptides *in vitro* and *in vivo*.**
13. AUGUSTYNS K, BAL G, THONUS G *et al.*: The unique properties of dipeptidyl-peptidase IV (DPP IV/CD26) and the therapeutic potential of DPP IV inhibitors. *Curr. Med. Chem.* (1999) 6:311-327.
    •• **The reader is referred to this review giving an excellent overview of the design and development of DPP IV inhibitors from the beginning of DPP IV research until 1998.**
14. VILLHAUER EB, COPPOLA GM, HUGHES TE: Chapter 19. DPP-IV inhibition and therapeutic potential. *Ann. Rep. Med. Chem.* (2001) 36:191-200.
15. MENTLEIN R, GALLWITZ B, SCHMIDT E: Dipeptidyl-peptidase IV hydrolyses gastric inhihitory polypeptide, glucagon-like peptide-1[7-36]amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur. J. Biochem.* (1993) 214:829-835.
16. KIEFFER TJ, MCINTOSH CHS, PEDERSON RA: Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 *in vitro* and *in vivo* by dipeptidyl peptidase IV. *Endocrinology* (1998) 136:3585-3596.
17. HANSEN L, DEACON CF, ORSKOV C, HOLST JJ: Glucagon-like peptide-1-[7-36]amide is transformed to glucagon-like peptide-1-[9-36]amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. *Endocrinology* (1999) 140:5356-5363.
18. DEACON CF, NAUCK MA, TOFT-NIELSEN M, PRIDAL L, WILLMS B, HOLST JJ: Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH_2-terminus in Type II diabetic patients and in healthy subjects. *Diabetes* (1995) 44:1126-1131.
19. HOLST JJ: Glucagon-like peptide-1, a gastrointestinal hormone with a pharmaceutical potential. *Curr. Med. Chem.* (1999) 6:1005-1017.
20. DRUCKER DJ: Development of glucagon-like peptide-1-based pharmaceuticals as therapeutic agents for the treatment of diabetes. *Curr. Pharm. Des.* (2001) 7:1399-1412.
21. DEACON CF, HUGHES TE, HOLST JJ: Dipeptidyl peptidase IV inhibition potentiates the insulinotropic effect of glucagon-like peptide 1 in the anesthetized pig. *Diabetes* (1998) 47:764-769.
22. MARGUET D, BAGGIO L, KOBAYASHI T *et al.*: Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proc. Nat. Acad. Sci. USA* (2000) 97:6874-6879.
23. NAGAKURA T, YASUDA N, YAMAZAKI K *et al.*: Improved glucose tolerance via enhanced glucose-dependent insulin secretion in dipeptidyl peptidase IV-deficient fischer rats. *Biochem. Biophys. Res. Comm.* (2001) 284:501-506.
24. YASUDA N, NAGAKURA T, YAMAZAKI K, INOUE T, TANAKA I: Improvement of high fat-diet-induced insulin resistance in dipeptidyl peptidase IV-deficient Fischer rats. *Life Sci.* (2002) 71:227-238.
25. SEDO A, MALIK R: Dipeptidyl peptidase IV-like molecules: homologous proteins or homologous activities? *Biochim. Biophys. Acta* (2001) 1550:107-116.
26. SCHÖN E, BORN I, DEMUTH HU *et al.*: Dipeptidyl peptidase IV in the immune system: effects of specific enzyme inhibitors on activity of dipeptidyl peptidase IV and proliferation of human lymphocytes. *Biol. Chem. Hoppe Seyler* (1991) 372:305-311.
    • **One of the first papers describing the effects of DPP IV inhibitors.**
27. AUGUSTYNS K, LAMBEIR AM, BORLOO M *et al.*: Pyrrolidides: synthesis and structure–activity relationship as inhibitors of dipeptidyl peptidase IV. *Eur. J. Med. Chem.* (1997) 32:301-309.
28. ASHWORTH DM, ATRASH B, BAKER GR *et al.*: 2-cyanopyrrolidides as potent, stable inhibitors of dipeptidyl peptidase IV. *Bioorg. Med. Chem. Lett.* (1996) 6:1163-1166.
    • **This paper describes an extensive structure–activity relationship of aminoacyl pyrrolidides and 2-cyanopyrrolidides as DPP IV inhibitors.**
29. LI J, WILK E, WILK S: Aminoacylpyrrolidine-2-nitriles: potent and stable inhibitors of dipeptidyl-peptidase IV (CD 26). *Arch. Biochem. Biophys.* (1995) 323:148-154.
    •• **The first paper on 2-cyanopyrrolidides as DPP IV inhibitors.**

30. SUDRE B, BROQUA P, WHITE RB *et al.*: Chronic inhibition of circulating dipeptidyl peptidase IV by FE 999011 delays the occurrence of diabetes in male Zucker diabetic fatty rats. *Diabetes* (2002) 51:1461-1469.

31. ASHWORTH DM, ATRASH B, BAKER GR *et al.*: 4-cyanothiazolididies as very potent, stable inhibitors of dipeptidyl peptidase IV. *Bioorg. Med. Chem. Lett.* (1996) 6:2745-2748.

32. GUTHEIL WG, BACHOVCHIN WW: Separation of L-Pro-DL-boroPro into its component diastereomers and kinetic analysis of their inhibition of dipeptidyl peptidase IV. *Biochemistry* (1993) 32:8723-8731.

33. FLENTKE GR, MUNOZ E, HUBER BT, PLAUT AG, KETTNER CA, BACHOVCHIN WW: Inhibition of dipeptidyl aminopeptidase IV (DP-IV) by Xaa–boroPro dipeptides and use of these inhibitors to examine the role of DP-IV in T cell function. *Proc. Natl. Acad. Sci. USA* (1991) 88:1556-1559.

34. DEMUTH HU, NEUMANN U, BARTH A: Reactions between dipeptidyl peptidase IV and diacyl hydroxylamines: mechanistic investigations. *J. Enzym. Inhibit.* (1989) 2:239-248.

35. BODUSZEK B, OLEKSYSZYN J, KAM CM, SELZLER J, SMITH RE, POWERS JC: Dipeptide phosphonates as inhibitors of dipeptidyl peptidase IV. *J. Med. Chem.* (1994) 37:3969-3976.

36. LAMBEIR AM, BORLOO M, DE MEESTER I *et al.*: Dipeptide-derived diphenyl phosphonate esters: mechanism-based inhibitors of dipeptidyl peptidase IV. *Biochim. Biophys. Acta* (1996) 1290:76-82.

37. DE MEESTER I, BELYAEV A, LAMBEIR AM *et al.*: *In vivo* inhibition of dipeptidyl peptidase IV activity by pro-pro-diphenyl-phosphonate (Prodipine). *Biochem. Pharmacol.* (1997) 54:173-179.

38. STÖCKEL MA, MRESTANI-KLAUS C, STIEBITZ B, DEMUTH HU, NEUBERT K: Thioxo amino acid pyrrolidides and thiazolidides: new inhibitors of proline specific peptidases. *Biochim. Biophys. Acta* (2000) 1479:15-31.

39. SENTEN K, VAN DER VEKEN P, BAL G *et al.*: Development of potent and selective dipeptidyl peptidase II inhibitors. *Bioorg. Med. Chem. Lett.* (2002) 12:2825-2828.

40. HUGHES TE, MONE MD, RUSSELL ME, WELDON SC, VILLHAUER EB: NVP-DPP728: (1-[[[2-[(5-cyanopyridin-2-yl)amino]ethyl]amino]acetyl]-2-cyano-(*S*)-ryrrolidine), a slow-binding inhibitor of dipeptidyl peptidase IV. *Biochemistry* (1999) 38:11597-11603.

•• **This paper describes the mechanism of action of one of the most potent and selective DPP IV inhibitors described today.**

41. VILLHAUER EB, BRINKMAN JA, NADERI GB *et al.*: 1-[2-[(5-cyanopyridin-2-yl)amino]-ethylamino]acetyl-2-(S)-pyrrolidine-carbonitrile: a potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties. *J. Med. Chem.* (2002) 45:2362-2365.

42. HUGHES TE, RUSSELL ME, BOLOGNESE L: NVP-LAF237, a highly selective and long-acting dipeptidyl peptidase IV inhibitor. *Diabetes* (2002) 51(Suppl.2):A67.

43. ZHAO K, LIM DS, FUNAKI T, WELCH JT: Inhibition of dipeptidyl peptidase IV (DPP IV) by 2-(2-amino-1-fluoro-propylidene)-cyclopentanecarbonitrile, a fluoroolefin containing peptidomimetic. *Bioorg. Med. Chem.* (2003) 11:207-215.

44. LIN J, TOSCANO PJ, WELCH JT: Inhibition of dipeptidyl peptidase IV by fluoroolefin-containing N-peptidyl-O-hydroxylamine peptidomimetics. *Proc. Natl. Acad. Sci. USA* (1998) 95:14020-14024.

45. BELYAEV A, ZHANG XM, AUGUSTYNS K *et al.*: Structure–activity relationship of diaryl phosphonate esters as potent irreversible dipeptidyl peptidase IV inhibitors. *J. Med. Chem.* (1999) 42:1041-1052.

46. NO AUTHORS LISTED: BDPX. *Drug Data Report* (2002) 24:620.

47. YAMADA M, OKAGAKI C, HIGASHIJIMA T, TANAKA S, OHNUKI T, SUGITA T: A potent dipeptide inhibitor of dipeptidyl peptidase IV. *Bioorg. Med. Chem. Lett.* (1998) 8:1537-1540.

48. OHNUKI T, YAMADA M, SUGITA T: Novel dipeptidyl peptidase IV inhibitors with antiarthritic effects. *Drugs of the Future* (1999) 24:665-670.

49. COPPOLA GM, ZHANG YL, SCHUSTER HF, RUSSELL ME, HUGHES TE: 1-aminomethylisoquinoline-4-carboxylates as novel dipeptidylpeptidase IV inhibitors. *Bioorg. Med. Chem. Lett.* (2000) 10:1555-1558.

50. PAULY RP, DEMUTH HU, ROSCHE F *et al.*: Improved glucose tolerance in rats treated with the dipeptidyl peptidase IV (CD26) inhibitor ile-thiazolidide. *Metabolism* (1999) 48:385-389.

51. PEDERSON RA, WHITE HA, SCHLENZIG D, PAULY RP, MCINTOSH CHS, DEMUTH HU: Improved glucose tolerance in Zucker fatty rats by oral administration of the dipeptidyl peptidase IV inhibitor isoleucine thiazolidide. *Diabetes* (1998) 47:1253-1258.

52. POSPISILIK JA, STAFFORD SG, DEMUTH HU *et al.*: Long-term treatment with the dipeptidyl peptidase IV inhibitor P32/98 causes sustained improvements in glucose tolerance, insulin sensitivity, hyperinsulinemia, and β-cell glucose responsiveness in VDF (*fa/fa*) Zucker rats. *Diabetes* (2002) 51:943-950.

53. SORBERA LA, REVEL L, CASTANER J: P32/98. Antidiabetic, dipeptidyl-peptidase IV inhibitor. *Drugs of the Future* (2001) 26:859-864.

54. DEACON CF, DANIELSEN P, KLARSKOV L, OLESEN M, HOLST JJ: Dipeptidyl peptidase IV inhibition reduces the degradation and clearance of GIP and potentiates its insulinotropic and antihyperglycemic effects in anesthetized pigs. *Diabetes* (2001) 50:1588-1597.

55. AHREN B, HOLST JJ, MARTENSSON H, BALKAN B: Improved glucose tolerance and insulin secretion by inhibition of dipeptidyl peptidase IV in mice. *Eur. J. Pharmacol.* (2000) 404:239-245.

56. BALKAN B, KWASNIK L, MISERENDINO R, HOLST JJ, LI X: Inhibition of dipeptidyl peptidase IV with NVP-DPP728 increases plasma GLP-1 [7-36 amide] concentrations and improves oral glucose tolerance in obese Zucker rats. *Diabetologia* (1999) 42:1324-1331.

57. AHREN B, SIMONSSON E, LARSSON H *et al.*: Inhibition of dipeptidyl peptidase IV improves metabolic control over a 4-week study period in Type 2 diabetes. *Diabetes Care* (2002) 25:869-875.

• **This paper describes the potential application of a DPP IV inhibitor in Type 2 diabetic patients.**

58. DEACON CF, WAMBERG S, BIE P, HUGHES TE, HOLST JJ: Preservation of active incretin hormones by inhibition of dipeptidyl peptidase IV suppresses meal-induced incretin secretion in dogs. *J. Endocrinol.* (2002) 172:355-362.

59. REIMER MK, HOLST JJ, AHREN B: Long-term inhibition of dipeptidyl peptidase IV improves glucose tolerance and preserves islet function in mice. *Eur. J. Endocrinol.* (2002) 146:717-727.

60. MITANI H, TAKIMOTO M, HUGHES TE, KIMURA M: Dipeptidyl peptidase IV inhibition improves impaired glucose tolerance in high-fat diet-fed rats: study using a Fischer 344 rat substrain deficient in its enzyme activity. *Jpn. J. Pharmacol.* (2002) 88:442-450.

61. MITANI H, TAKIMOTO M, KIMURA M: Dipeptidyl peptidase IV inhibitor NVP-DPP728 ameliorates early insulin response and glucose tolerance in aged rats but not in aged Fischer 344 rats lacking its enzyme activity. *Jpn. J. Pharmacol.* (2002) 88:451-458.

62. RASMUSSEN HB, BRANNER S, WIBERG FC, WAGTMANN N: Crystal structure of human dipeptidyl peptidase IV/CD26 in complex with a substrate analog. *Nat. Struct. Biol.* (2003) 10:19-25.

•• **The very recent publication of the crystal structure of DPP IV will certainly enhance the rational development of new DPP IV inhibitors.**

Patents

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

101. FERRING BV: WO9515309 (1995).

•• **The first patent on cyanopyrrolidides as DPP IV inhibitors. Comparative inhibition data with other compounds are given.**

102. PROBIODRUG: WO9967278 (1999).

103. NOVARTIS AG: US6107317 (2000).

104. NOVARTIS AG: US6124305 (2000).

•• **A patent describing a large series of N-substituted-glycyl-2-cyanopyrrolidides including biochemical and biological data.**

105. NOVARTIS AG: US6110949 (2000).

106. NOVARTIS AG: US6166063 (2000).

107. FERRING BV: WO0140180A2 (2001).

108. FERRING BV: WO0181304A1 (2001).

109. NOVO NORDISK AS: WO0155105 (2001).

110. BRISTOL-MYERS SQUIBB CO.: WO0168603 (2001).

111. TAISHO PHARM. CO. LTD: WO0238541 (2002).

112. MITSUBISHI PHARMA.: WO0214271 (2002).

113. TANABE SEIYAKU: WO0230890, WO0230891 (2002).

114. KYOWA HAKKO KOGYO CO. LTD: WO0251836 (2002).

115. POINT THERAPEUTICS, INC.: WO9962914 (1999).

116. FONDATECH BENELUX NV: WO9946272 (1999).

117. NOVO NORDISK AS: WO0202560 (2002).

• **The first patent describing non-peptide DPP IV inhibitors. Unfortunately no biochemical or biological data are included.**

Affiliation

Koen Augustyns†, Pieter Van der Veken, Kristel Senten & Achiel Haemers
†Author for correspondence
Department of Medicinal Chemistry, University of Antwerp, Universiteitsplein 1, B-2610, Antwerpen, Belgium
Tel: +32 (0)3820 27 03; Fax: +32 (0)3820 27 39; E-mail: Koen.Augustyns@ua.ac.be

Expert Opinion

1. Introduction to incretin biology
2. Dipeptidyl peptidase IV
3. Dipeptidyl peptidase IV inhibitors
4. Expert opinion

Ashley Publications
www.ashley-pub.com

Therapeutic potential of dipeptidyl peptidase IV inhibitors for the treatment of type 2 diabetes

Daniel J Drucker
Department of Medicine, Banting and Best Diabetes Centre, Toronto General Hospital, University of Toronto, Toronto, Ontario, M5G 2C4, Canada

Incretins are peptide hormones, exemplified by glucose-dependent insulinotropic peptide and glucagon-like peptide 1 that are released from the gut in response to nutrient ingestion and enhance glucose-stimulated insulin secretion. Incretin action is terminated due to N-terminal cleavage of the peptides by the aminopeptidase dipeptidyl peptidase IV (DPP-IV). Hence, inhibition of glucose-dependent insulinotropic peptide and glucagon-like peptide 1 degradation via reduction of DPP-IV activity represents an innovative strategy for enhancing incretin action *in vivo*. This review summarises the biology of incretin action, the structure, expression and pleiotropic biological activities of DPP-IV and provides an overview of the rationale, potential merits and theoretical pitfalls in the development of DPP-IV inhibitors for the treatment of type 2 diabetes.

Keywords: diabetes, drugs, enzyme inhibitors, GIP, GLP-1, glucagon-like peptides, glucose, incretin, inhibitor, peptidase, peptide

Expert Opin. Investig. Drugs (2003) 12(1):87-100

1. Introduction

Incretins are gut peptides, predominantly glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide 1 (GLP-1), which are released from the gastrointestinal tract in response to nutrient ingestion and promote nutrient assimilation via potentiation of glucose-dependent insulin secretion. Incretins, particularly GLP-1, also function in part by contributing to the neurohormonal signals emanating from the distal gut, the 'ileal brake', which regulate the rate through which nutrients transit along the GI tract. The available evidence suggests that enhancement of incretin action may be useful for lowering blood glucose in subjects with type 2 diabetes mellitus. Nevertheless, subjects with type 2 diabetes or obesity may exhibit a diminution in the secretion of endogenous incretins, particularly GLP-1, following food ingestion [1,2]. Furthermore, incretin action is rapidly terminated via the action of dipeptidyl peptidase IV (DPP-IV), which inactivates both GIP and GLP-1 via cleavage at the position 2 alanine. This review provides an overview of incretin and DPP-IV biology, with a focus on critical evaluation of the issues surrounding the use of DPP-IV inhibitors for the treatment of type 2 diabetes.

1.1 Glucose-dependent insulinotropic peptide actions

GIP is a 42 amino acid peptide originally characterised as an active component of intestinal extract that inhibited gastric acid secretion, hence its original designation as a gastric inhibitory polypeptide. Subsequent studies demonstrated that GIP exhibited potent insulinotropic properties [3], leading to its concurrent designation as a glucose-dependent insulinotropic polypeptide. GIP directly enhances insulin secretion through a specific GIP receptor expressed on islet β cells [4]. The physio-

 ISSN 1354-3784

logical importance of GIP action has been delineated in studies using peptide antagonists, GIP receptor knockout mice or antisera directed against the GIP receptor. Acute impairment of GIP action results in defective glucose-stimulated insulin secretion in rats and mice [5,6]. Similarly, mice with targeted disruption of the GIP receptor gene (GIPR-/-) exhibit normal fasting glucose but impaired glucose clearance and reduced insulin secretion following oral glucose challenge [7]. GIP receptors are also expressed on adipocytes, where GIP action may promote fatty acid synthesis and lipid accumulation [8]. Intriguingly, GIPR-/- mice are resistant to weight gain and develop less extensive glucose intolerance following months of high fat feeding, yet exhibit normal feeding behaviour, enhanced fuel oxidation and increased metabolic rate [9]. Furthermore, a double mutant ob:ob/GIPR-/- mouse exhibits less weight gain and relatively improved glucose tolerance compared to the ob:ob mouse alone. These unexpected findings suggest that GIP receptor antagonists merit further analysis in the setting of nutrient-induced obesity and glucose intolerance.

Although GIP stimulates insulin secretion in normal rodents and human subjects, its insulinotropic actions are markedly attenuated in experimental diabetes [10], perhaps due in part to decreased GIP receptor expression and/or function. Similarly, GIP infusion in human subjects with type 2 diabetes does not increase insulin secretion to the same extent seen in normal subjects [11-14], and normoglycaemic relatives of subjects with type 2 diabetes exhibit decreased GIP responsivity [15]. These findings suggest that type 2 diabetes may be associated with both genetic and acquired resistance to GIP action. Hence, there remains comparatively reduced enthusiasm for the use of GIP agonists alone in the treatment of type 2 diabetes, although recent reports suggest that modified protease-resistant GIP receptor agonists [16] may exert glucose-lowering effects in subjects with type 2 diabetes.

1.2 Glucagon-like peptide 1 actions *in vivo*

Two equipotent bioactive forms of GLP-1, GLP-1(7-36)amide and GLP-1(7-37), are liberated from proglucagon in enteroendocrine L cells and secreted in response to nutrient intake. Meal-stimulated GLP-1 secretion appears attenuated in human subjects with type 2 diabetes [1,17]. GLP-1 levels fall rapidly following postprandial excursion, and clearance reflects the actions of the kidney [18,19], enzymatic inactivation by DPP-IV [20-22] and, to a lesser extent, neutral endopeptidase (NEP) 24.11 [23]. GLP-1 actions on the islet β cell include stimulation of glucose-dependent insulin secretion [24-26] and induction of glucose competence [27]. GLP-1 also increases insulin gene promoter activity and insulin biosynthesis in cell lines [28,29] and in rodents [30]. GLP-1 also lowers blood glucose via inhibition of gastric emptying [31], thereby attenuating the rate of nutrient entry into the circulation [32], and inhibition of glucagon secretion from islet α cells, probably via effects on insulin or somatostatin secretion [33].

GLP-1 directly stimulates glucose-dependent insulin secretion via an increase in β-cell cAMP [24], through both protein kinase A-dependent and independent mechanisms, with activation of signalling through small G proteins contributing to control of insulin exocytosis [34]. GLP-1 receptor (GLP-1R) activation also promotes calcium mobilisation [35] and closure of the ATP-sensitive K_{ATP} channel [36]. Furthermore, genetic disruption of SUR channel activity in mice is associated with resistance to the insulinotropic actions of both GLP-1 and GIP.

GLP-1 administration to normal or diabetic rodents induces β-cell proliferation [37] and islet neogenesis, leading to an increase in β-cell mass [38,39]. Furthermore, treatment of pancreatic exocrine cell lines with GLP-1R agonists induces a programme of endocrine differentiation associated with molecular features of functional β cells capable of glucose-stimulated insulin secretion [40,41]. The cellular signals coupling GLP-1R activation to islet cell growth appear to include activation of the mitogen-activated protein kinase (MAPK) pathway, protein kinase C and the transcription factor PDX-1 [42]. These observations raise the possibility that GLP-1 may be able to preserve or restore deteriorating β-cell function in type 2 diabetics in part via islet regeneration and augmentation of functional β-cell mass.

GLP-1 also stimulates secretion of hypothalamic-pituitary hormones [43-45] and induces potent anorexic effects following intracerebroventricular administration in rats and mice [46,47]. Furthermore, chronic administration of GLP-1 analogues is associated with weight loss in experimental rodent and primate models of diabetes [48,49], and peripheral GLP-1 administration induces satiety and reduces meal consumption in normal, obese and diabetic human subjects [50,51].

1.3 Essential physiological actions of glucagon-like peptide 1

Experiments using GLP-1R antagonists and characterisation of GLP-1R null (GLP-1R-/-) mice have revealed essential physiological actions dependent on GLP-1R signalling. Acute administration of the GLP-1R antagonist exendin(9-39) increases fasting glycaemia and impairs glucose clearance following glucose loading, in association with decreased levels of circulating insulin, in both rodents and human subjects [5,52-55]. Similarly, GLP-1R-/- mice exhibit mild fasting hyperglycaemia and impaired glucose clearance following either oral or intraperitoneal glucose loading [56]. Although chronic intracerebroventricular administration of exendin (9-39) increased food intake and weight gain in rats [57], GLP-1R-/- mice in the CD1 genetic background are lean and do not develop obesity even following prolonged high fat feeding [58]. Moreover, GLP-1R-/- mice exhibit only modest defects in islet size and topography [59] and develop appropriate islet hyperplasia and upregulation of insulin gene expression in response to obesity and insulin resistance [60]. Hence, genetic loss of GLP-1R signalling in the mouse does not produce major perturbations in β-cell growth or insulin biosyn-

thesis, perhaps due to upregulation of compensatory mechanisms, such as enhanced secretion of and sensitivity to GIP [61].

1.4 Glucagon-like peptide 1 actions in normal and diabetic human subjects

Consistent with preclinical findings, short-term GLP-1 infusion in normal subjects potentiates glucose-dependent insulin secretion, inhibits glucagon secretion and gastric emptying and produces short-term satiety leading to reduction in food intake [13,50,62,63]. GLP-1 administration to subjects with type 2 diabetes also lowers glycaemia following subcutaneous or intravenous short-term administration [64-67]. GLP-1 also enhances β-cell responsivity to sulfonylurea agents in subjects with type 2 diabetes [68]. The importance of gastric emptying and glucagon secretion for GLP-1 action is exemplified by studies demonstrating glucose-lowering effects of GLP-1 in patients with type 1 diabetes mellitus [62,69].

Native GLP-1 is rapidly degraded to inactive GLP-1(9-37) or GLP-1(9-36)amide [21,22,70]. The plasma half-lifes of GLP-1 (7-36) amide and GLP-1(7-37) as assessed by exogenous infusion of the peptides in human subjects are similar, 5.3 ± 0.4 versus 6.1 ± 0.8 min, respectively, and the metabolic clearance rates of the two biologically active GLP-1 molecules were also comparable (14.6 ± 2.4 versus 12.2 ± 1 pmol/kg x min) [71]. The short duration of endogenous GLP-1 action, taken together with subsequent data demonstrating that a continuous 24-h infusion of GLP-1 was superior to a similar but shorter 16-h infusion for lowering of blood glucose in poorly controlled diabetic subjects [72], provides a sound rationale for development of longer-acting degradation-resistant GLP-1 analogues or continuous infusion approaches for the treatment of patients with type 2 diabetes [73].

1.5 Treatment of type 2 diabetes with glucagon-like peptide 1 receptor agonists

Following observations that short-term 24 - 48 h GLP-1 infusions lowered blood glucose in diabetic subjects [74,75], several studies examined the metabolic consequences of longer periods of GLP-1 infusion using the native peptide. A 3-week infusion of GLP-1 in six subjects with type 2 diabetes lowered meal-related glycaemic excursion, increased plasma insulin and decreased plasma glucagon in the postprandial period, with no evidence for tachyphylaxis at the end of the 3-week treatment period [76]. A longer 6-week continuous subcutaneous GLP-1 infusion study in 20 subjects with type 2 diabetes demonstrated significant improvements in mean plasma glucose, fructosamine, haemoglobin (Hb)A1c, fasting and postprandial free fatty acids, with reduced gastric emptying, weight gain and improved β-cell function in the GLP-1-treated subjects [51]. Hence, native GLP-1, if chronically delivered via a continuous infusion strategy, appears highly effective for the treatment of type 2 diabetes.

To circumvent the need for continuous GLP-1 administration, considerable effort has been directed towards the generation of long-acting degradation-resistant GLP-1 analogues suitable for once- or twice-daily administration. Several GLP-1R agonists are currently in clinical trials including the lizard peptide exendin-4 [77] and a fatty acid derivatised DPP-IV-resistant analogue, NN2211 [49]. Intravenous infusion of exendin-4 in normal [63] and diabetic subjects [78] lowers fasting and postprandial plasma glucose [63], stimulates insulin secretion, reduces levels of circulating glucagon and inhibits gastric emptying and food intake. Similarly, subcutaneous administration of NN2211 results in a plasma drug half-life of ~ 12.6 h [79] and lowers both fasting and postprandial glycaemia via effects on insulin and glucagon secretion and gastric emptying [80].

2. Dipeptidyl peptidase IV

2.1 Structure and expression of DPP IV

DPP-IV, also known as the lymphocyte cell surface protein CD26, is a widely expressed glycoprotein that exhibits three principal biological activities: in humans, it functions as an adenosine deaminase (ADA)-binding protein; it contributes to extracellular matrix binding; and of direct relevance to this review, it exhibits post proline or alanine peptidase activity, thereby inactivating or in some cases generating biologically active peptides via cleavage at the N-terminal region after X-proline or X-alanine (**Box 1**) [81,82]. DPP-IV exists as a membrane bound 110 kDa glycoprotein that is catalytically active as a dimer, whose structure is reasonably well conserved across different mammalian species. The human DPP-IV gene contains 26 exons, is localised to the long arm of chromosome 2 and intriguingly, is localised adjacent to the proglucagon gene which encodes GLP-1 and GLP-2, principal substrates for DPP-IV. The human DPP-IV cDNA encodes a predicted protein of 766 amino acids, with 6 amino acids in the cytoplasm, 22 residues spanning the plasma membrane and 738 amino acids comprising the extracellular domain. DPP-IV also exists as a soluble circulating form of ~ 100 kDa, which retains both adenosine deaminase binding and enzymatic activity and the N-terminal amino acid of the soluble form appears to be Ser39 [83,84]. Consistent with the classical serine protease consensus motif of G-X-S-X-G, the corresponding sequence in DPP-IV is G-W-S-Y-G, with selected mutations in a novel catalytic triad of Ser624, Asp702 and His734 abolishing catalytic activity of the murine molecule [85].

DPP-IV is a widely expressed enzyme present on cells in most tissues, including the kidney, gastrointestinal tract, biliary tract and liver, placenta, uterus, prostate, skin and, of potential relevance to the clinical use of inhibitors, lymphocytes (immune function) and endothelial cells (inactivation of circulating peptides) [86-88]. Furthermore, the expression of DPP-IV in specific tissues or as a circulating soluble form, is widely modulated in the setting of specific diseases or tissue injury and inflammation, as reviewed in [81,89-92] and summarised in **Table 1**.

Box 1. Putative substrates for dipeptidyl peptidase IV.

Xaa-Pro
- Tyr-Melanostatin
- Endomorphin-2
- Enterostatin
- B-Casomorphin
- Trypsinogen pro-peptide
- Bradykinin
- Substance P
- CLIP
- Gastrin-releasing peptide (GRP)
- Neuropeptide Y (NPY)
- Peptide YY (PYY)
- Aprotinin
- RANTES
- Granulocyte chemotactic protein-2 (GCP-2)
- Stromal cell-derived factor 1a (SDF-1a)
- Stromal cell-derived factor 1b (SDF-1b)
- Macrophage-derived chemokine (MDC)
- Monocyte chemoattractant protein 1 (MCP-1)
- Monocyte chemoattractant protein 2 (MCP-2)
- Monocyte chemoattractant protein 3 (MCP-3)
- Eotaxin
- Interferon-inducible protein 10 (IP-10)
- Insulin-like growth factor (IGF-I)
- Procolipase
- Interleukin-2 (IL-2)
- Interleukin-1b (IL-1b)
- α1-Microglobulin
- Prolactin
- Trypsinogen
- Human chorionic gonadotrophin (HCG)

Xaa-Ala
- Peptide histidine-methionine (PHM)
- Glucose-dependent insulinotropic peptide (GIP)
- Growth hormone-releasing hormone (GRH)
- Glucagon-like peptide 1 (GLP-1)
- Glucagon-like peptide 2 (GLP-2)

Box 2. Experimental diseases or conditions modified by DPP-IV inhibition.

- Diabetes
- Experimental encephalomyelitis
- Murine abortion
- Sensitivity to chemotherapy
- Invasion, growth and migration of cancer cells
- Keratinocyte DNA synthesis
- Experimental nephritis
- Experimental arthritis

DDP-IV: Dipeptidyl peptidase IV.

2.2 CD26/DPP-IV and normal immune function

Originally identified as a lymphocyte cell surface ADA-binding protein with costimulatory activity, CD26 expression and activity are increased following T-cell activation and distinct subpopulations of CD26^{bright} T cells have been identified that subserve multiple functions, including antigen recall, immunoglobulin synthesis and activation of cytotoxic T cells [82]. CD26 associates with other lymphocyte cell surface molecules, including the chemokine receptor CXCR4, ADA and CD45 [89,93], and mAbs against CD26 promote aggregation of both CD26 and CD45 into lymphocyte lipid rafts. Furthermore, CD26 directly binds to the cytoplasmic domain of CD45, providing a mechanism for engagement of specific signal transduction pathways leading to IL-2 production [94], a common downstream event secondary to CD26 activation. Conversely, interleukin induces CD26 expression on a subset of human natural killer lymphocytes [95]. Activation of lymphocyte CD26 leads to increases in intracellular calcium, tyrosine phosphorylation of multiple substrates and cell proliferation [96,97]. CD26 undergoes mannose-6 phosphorylation leading to interaction with the mannose-6-phosphate/insulin-like growth Factor II receptor (M6P/IGFII) receptor following T cell activation [98]. Soluble CD26 also interacts with the (M6P/IGFII) and enhances transendothelial T-cell migration, an effect that requires its DPP-IV enzymatic activity [99].

The majority of experiments assessing lymphocyte CD26 activity use specific antibodies for CD26 activation; whether the enzymatic peptidase activity of CD26 is involved in or required for multiple aspects of lymphocyte signalling has not always been conclusively determined [93]. Experiments carried out with mutant soluble CD26 molecules have demonstrated the importance of DPP-IV enzymatic activity for enhancement of T-cell proliferation and induction of monocyte CD86 expression [100]. Similarly, antiCD26 mAbs inhibit T-cell growth and proliferation via induction of G1/S arrest, effects which are dependent on the enzymatic function of CD26 [101]. Interpretation of data obtained from experiments using specific DPP-IV inhibitors to examine lymphocyte function is complicated by the specificity of the inhibitor employed. However, DPP-IV inhibition has been shown to modify T- and B-cell proliferation and cytokine production, as reviewed in [82]. In contrast, analyses of cells from the CD26/DPP-IV mutant Fischer 344 rat or the CD26/DPP-IV knockout mouse have not yet revealed major defects in lymphocyte activation or immune function [102,103]. The available evidence suggests that the enzymatic activity of DPP-IV may not be essential for many of the T-cell activating or costimulatory properties attributed to CD26. However, not all experiments have used both wild-type and mutant CD26 molecules to examine this specific question.

2.3 CD26/DPP-IV activity and disease

DPP-IV activity is increased in patients with cholestatic hepatobiliary disease [104], hepatitis-C-associated liver injury [105] or osteoporosis [106], and in T cells from patients with multiple sclerosis [107] (Table 1). CD26 expression and activity may

Table 1. Human diseases characterised by changes in DPP-IV activity.

Human DPP-IV activity	
Increased	**Decreased**
Rheumatoid arthritis	AIDS
Multiple sclerosis	Down's syndrome
Graves' disease	Common variable
Hashimoto's thyroiditis	hypogammaglobulinemia
Sarcoidosis	Vasculitis/systemic lupus erythematosis/
Psychological stress	rheumatoid arthritis
Cancer	Cancer
	Anorexia/bulimia
	Depression
	Pregnancy

DPP-IV: Dipeptidyl peptidase IV.

be reduced in T-cell subsets from patients with active HIV [108,109], but increased in HIV-infected subjects with immune reconstitution [110].

In contrast, serum DPP-IV activity is decreased during pregnancy [111], in subjects with active Crohn's disease [112], major depressive illness [113,114], eating disorders [115], active systemic lupus erythematosis [116] or rheumatoid arthritis [117]. Similarly, serum DPP-IV activity is decreased in subjects with active Wegener's granulomatosis, Churg-Strauss syndrome and microscopic polyangiitis, with levels increasing in patients with disease remission [118]. Of potential clinical relevance to diabetes therapeutics, DPP-IV activity is significantly reduced in hypertensive patients treated with angiotensin-converting enzyme (ACE) inhibitors when measured during an episode of drug-associated angioedema [119].

Altered CD26/DPP-IV expression has also been associated with specific cancers, including well differentiated thyroid cancer [120,121] and prostate cancer [122]. Levels are also altered in some patients with colon cancer [123] and oral cancer [124].

2.4 DPP-IV enzymatic activity and physiological peptide substrates

A large number of potential peptide substrates for DPP-IV have now been identified, as summarised in **Box 1** and reviewed in [81]. For many of these substrates, evidence implicating a role for DPP-IV in peptide cleavage derives from pharmacological kinetic studies demonstrating that incubation of the peptide and purified enzyme *in vitro* produces peptide cleavage at the N-terminus [125,126]. Whether this line of pharmacological evidence necessarily implies a physiological role for DPP-IV as an essential regulator of peptide activity *in vivo* remains unclear [127]. For example, incubation of 29 amino acid glucagon with purified DPP-IV yields glucagon(3-29) and glucagon(5-29) [128,129] and immunoreactive DPP-IV has been colocalised with glucagon in islet A cell granules [130]. However, increased levels of intact glucagon have not been demonstrated in CD26-/- mice or Fischer 344 DPP-IV mutant rats or following

Box 3. Criteria for establishing a physiological role for DPP-IV in substrate cleavage.

Cleavage of the peptide by purified enzymatically active DPP-IV *in vitro*
Peptide degradation in vitro inhibited by DPP-IV inhibitors
Altered ratio of intact to degraded peptide following acute DPP-IV inhibitor administration to normal animals or humans *in vivo*
Altered ratio of intact to degraded peptide substrate in mice or rats with genetic inactivation of DPP-IV

DPP-IV: Dipeptidyl peptidase IV

administration of DPP-IV inhibitors to normal rodents or humans and blood glucose is uniformly lower following administration of DPP-IV inhibitors *in vivo*. Hence, establishment of criteria, as suggested in **Box 3** requiring demonstration that levels of non-cleaved putative DPP-IV substrates are increased in genetic models of DPP-IV deficiency and following administration of DPP-IV inhibitors provides a more rigorous definition for establishing whether specific peptides are physiological (as opposed to pharmacological) targets of DPP-IV enzymatic activity.

The principal known peptide substrates considered major targets of DPP-IV inhibitors when used for the treatment of diabetes are GLP-1 and GIP. Following pharmacological demonstration that purified DPP-IV cleaves both these peptides at the position 2 alanine [20,21], infusion of radiolabelled GIP and GLP-1 into DPP-IV deficient rats revealed almost complete absence of the predicted degradation products, GIP(3-42) and GLP-1(9-36)NH_2. Concomitant experiments demonstrated that GIP(3-42) and GLP-1(9-36)NH_2 represented the principal degradation products present in human plasma in both the fasting and postprandial states [22]. The degradation of intact GLP-1 occurs rapidly, as GLP-1(9-36)NH_2 represents > 50% of detectable immunoreactive GLP-1 released from the isolated perfused porcine ileum [131], with the proportion of intact to N-terminal cleaved GLP-1 greatly increased following administration of DPP-IV inhibitors [131]. Similarly, studies employing structurally unique DPP-IV inhibitors confirmed that increased circulating levels of intact GLP-1 and GIP were detectable following inhibitor administration [132-135]. Furthermore, the proportion of intact to N-terminally degraded GLP-1 and GIP is increased in mice [103] and rats [136] with inactivating mutations of the DPP-IV gene. Hence, both GLP-1 and GIP satisfy multiple criteria (**Box 3**) for designation as physiological peptide substrates of DPP-IV *in vivo*. Although GLP-1(9-36)NH_2 is a weak pharmacological antagonist at the GLP-1R, it does not seem to function as a physiologically relevant antagonist *in vivo* [137].

2.5 DPP-IV inhibitors and experimental disease

Given the pleiotropic activities of and potential substrates for DPP-IV, the effect of activating and more commonly inhibit-

ing DPP-IV activity has been examined in different experimental models, including neoplastic cell growth. CD26 binds to extracellular matrix components including collagen and fibronectin, potentially modifying cell adhesion, migration and metastatic behaviour. The potential relationship between CD26 expression or activity in neoplastic cells and clinical behaviour of specific tumours, is complex and highly tumour cell-specific. Human T-cell leukaemia Jurkat cells transfected with wild-type DPP-IV or mutant DPP-IV devoid of ADA binding yet retaining enzymatic activity, exhibit increased sensitivity to the cytotoxic effects of doxorubicin [138]. Similarly, soluble CD26 enhanced the growth inhibitory effects of doxorubicin *in vitro*. Consistent with the loss of DPP-IV expression during melanoma progression, inducible re-expression of DPP-IV led to loss of tumorigenicity in human melanoma cells, findings dependent on serine protease activity [139], whereas DPP-IV-transfected melanoma cells exhibited normal growth but reduced migration independent of the proteolytic activity of the enzyme [140]. DPP-IV expression in human ovarian cancer cell lines also correlates with reduced migration, invasion and decreased peritoneal dissemination in nude mice *in vivo* [141]. In contrast, inhibition of DPP-IV activity with diprotin A enhanced invasion of placental JEG-3 cells *in vitro* [142]. Paradoxically, the related membrane bound protease seprase or fibroblast-activating protein, promotes tumour growth and together with DPP-IV, forms a complex on the cell surface that participates in gelatin binding and degradation in migratory fibroblast cells *in vitro* [143]. Hence, the effects of DPP-IV expression and activity on cell growth, migration, invasion and tumorigenicity appear cell- and context-specific.

The importance of DPP-IV expression and activity has also been examined in experimental inflammatory disorders. Treatment of mice with the reversible DPP-IV inhibitor Lys[Z(NO[2])]-pyrrolidide decreased the extent and onset of adoptive transfer experimental autoimmune encephalomyelitis, effects mediated in part through upregulation of transforming growth factor (TGF)-β1 activity [144]. Similarly, DPP-IV inhibitors attenuated the extent of collagen- and alkyldiamine-induced arthritis in rats, and a mAb directed against CD26 suppressed experimental nephritis in rats in association with markedly reduced complement activation [145]. Local DPP-IV expression has been proposed as a modulator of substance-P-induced vasodilatation in the setting of chronic rhinosinusitis [146]. However, the importance of local versus systemic DPP-IV enzymatic activity for the development of inflammation-associated vasodilatation remains uncertain.

2.6 DPP-IV-related proteases and specificity of DPP-IV inhibitors

The term DPP-IV activity- and/or structure-homologues has been applied to describe the family of often structurally-related enzymes that exhibit overlapping enzymatic activity with DPP-IV [147]. Several recent reviews have summarised the features of mammalian endo- and exopeptidases capable of cleaving peptides at the N-terminal position 2 alanine or proline (**Box 1**) [81,147]. Hence, rigorous experimental proof is required to implicate an essential physiological role for a specific peptidase in cleavage of peptide substrates *in vivo*. The putative roles of DPP-IV in lymphocyte signalling, cell growth and migration and the importance of enzymatic activity for the cleavage of regulatory peptides have been evaluated with immunoneutralisation and genetic approaches. For example, the availability of rats or mice with inactivating mutations in the DPP-IV gene provides an opportunity to assess the essential or redundant role(s) of the DPP-IV gene in a broad variety of biological systems. Similarly, the binding of DPP-IV to human adenosine deaminase provides an approach for removal of the DPP-IV molecule from specific fluids or extracts, providing a non-genetic approach for the assessment of the biological importance of DPP-IV [84]. Furthermore, experiments employing mutant DPP-IV molecules in which the enzymatic activity of DPP-IV has been specifically inactivated are particularly useful for understanding the contributions of individual CD26 functional domains in a broad spectrum of CD26 biological activities.

In contrast, the use of 'specific' DPP-IV enzyme inhibitors alone to infer biological activities ascribed to DPP-IV is constrained by the difficulty inherent in validating the precise specificity of individual enzyme inhibitors.

3. Dipeptidyl peptidase IV inhibitors

3.1 DPP-IV inhibition and experimental models of type 2 diabetes

Considerable evidence from studies in rats, mice, dogs and human subjects attests to the concept and efficacy of using DPP-IV inhibitors for the treatment of diabetes [148], and has recently been reviewed [149,150]. The inhibitor valine pyrrolidide (Val-pyr) reduced porcine plasma DPP-IV activity by > 90% and decreased the degradation of intact GLP-1, both in the fasted state and following exogenous GLP-1 administration [151]. Infusion of glucose together with GLP-1 in the presence of Val-pyr produced a significant augmentation in levels of plasma insulin compared to GLP-1 infusion in the absence of the inhibitor [151]. The DPP-IV inhibitor isoleucine thiazolidide (Ile-thiazolidide) prevented N-terminal degradation of both GLP-1 and GIP in human serum, and oral administration of Ile-thiazolidide to both lean or obese Zucker fatty rats inhibited plasma DPP-IV activity, decreased glycaemic excursion and enhanced levels of circulating insulin following oral glucose loading [152]. In contrast, administration of the inhibitor alone without concomitant glucose loading had no effect on levels of fasting glucose or insulin in obese Zucker rats [152].

Analysis of rat plasma following administration of both Ile-thiazolidide and radiolabelled GLP-1 demonstrated that 70% inhibition of rat plasma DPP-IV activity markedly reduced the degradation of exogenous [125I]-labelled GLP-1(7-

$36)NH_2$ [132]. Furthermore Ile-thiazolidide reduced glycaemic excursion, enhanced levels of plasma insulin and prolonged the half-life of endogenous GLP-1(7-36)NH_2 released following intraduodenal glucose loading [132]. Similar results were obtained following administration of the inhibitor NVP-DPP728 to lean and obese Zucker rats, with enhanced insulin release and reduced glycaemic excursion detected in the inhibitor-treated rats, in association with markedly enhanced levels of intact GLP-1(7-36)NH_2 [133].

The glucose-lowering properties of Val-Pyr were subsequently examined in C57BL/6 mice following 5 weeks of high fat (58% total fat) feeding. Consistent with previous findings, Val-Pyr markedly augmented the plasma levels of GLP-1 following intravenous GLP-1 administration to normal C57BL/6 mice, and acute inhibitor administration decreased glycaemic excursion and increased levels of both insulin and GLP-1 following oral glucose loading in both high fat fed and control mice [153]. In contrast, Val-Pyr had no effect on glucose-stimulated insulin secretion from isolated islets *in vitro*. The importance of GIP as a substrate for DPP-IV inhibitors is illustrated by experiments in mice and pigs. Administration of Val-Pyr to GLP-1R knockout mice produces a glucose-lowering effect, suggesting that DPP-IV substrates independent of GLP-1 are also important for glucose clearance *in vivo* [103]. Similarly, Val-Pyr markedly reduces the N-terminal degradation of intact GIP and potentiates the insulinotropic actions of infused GIP in pigs [154].

More recent studies have examined the effects of chronic DPP-IV inhibitor administration in rodent models of type 2 diabetes. Oral administration of P32/98, 20 mg/kg b.i.d. for 3 months was associated with a progressive improvement in fasting glucose over the 12-week study period, in association with enhanced levels of glucose-stimulated insulin, a 12.5% decrease in relative body weight gain and improvements in insulin sensitivity as assessed at the end of the treatment period [155,156]. Inhibitor-treated rats exhibited enhanced insulin release following pancreatic perfusion with 8.8 mM glucose, increased insulin-stimulated adipose tissue glycogen synthase activity and increased insulin-stimulated methyl glucose uptake in soleus muscle strips [155]. The mechanism by which incretin hormones increase insulin sensitivity remains unclear, however, similar findings have been observed in human diabetic subjects treated with continuous GLP-1 infusion for 6 weeks [51]. Interestingly, despite the marked improvements in glucose homeostasis observed in inhibitor-treated rats, fasting levels of plasma DPP-IV activity were significantly increased in P32/98-treated rats. However, the precise source of increased circulating plasma DPP-IV remains unclear [155].

Chronic inhibition of DPP-IV activity has also been studied in Zucker diabetic fatty rats treated once- or twice-daily with the long-acting inhibitor FE 99901. This compound produced comparatively greater and sustained inhibition of plasma DPP-IV activity compared to similar doses of NVP-DPP728 after single dosing, and a 7-day treatment period with FE 99901 improved glucose tolerance in association with increased levels of glucose-stimulated insulin [157]. Chronic twice-daily treatment with FE 99901 for 25 days significantly delayed the deterioration in plasma glucose observed in control rats treated with vehicle alone, in association with a reduction in food intake and water consumption and modest but significant increases in the levels of circulating GLP-1. Furthermore, FE 99901-treated rats displayed significant reductions in levels of free fatty acids and triglycerides and increased pancreatic expression of the GLP-1R [157]. Twice-daily inhibitor administration was significantly more effective than once-daily treatment, attesting to the importance of sustained suppression of plasma and/or tissue DPP-IV activity for optimal glucose control.

The effect of an 8-week treatment period using NVP-DPP728 was examined in C57BL/6 mice fed a high fat diet [135]. Treatment was commenced at 5 weeks of age and NVP-DPP728 was added continuously in the drinking water at a concentration of 0.12 µmol/g body weight, resulting in marked suppression of plasma DPP-IV activity to < 5% of control values. Inhibitor-treated mice fed normal or high fat diets did not exhibit differences in body weight, but cumulative food intake was significantly reduced in high fat fed mice treated with NVP-DPP728 when assessed during the last week of the study period [135]. Glucose tolerance improved and both circulating insulin and GLP-1 levels increased following 8 weeks of inhibitor treatment in normal or high fat fed mice [135]. Furthermore, glucose-stimulated insulin secretion was improved in isolated islets from inhibitor-treated mice and islet size was smaller in mice treated with NVP-DPP728 [135]. Hence the available evidence from a variety of rodent models supports the efficacy of chronic DPP-IV inhibitor administration for the treatment of experimental type 2 diabetes.

3.2 DPP-IV inhibition and the treatment of human subjects with type 2 diabetes

Only limited information is currently available concerning the clinical efficacy of DPP-IV inhibitors in the treatment of human subjects with type 2 diabetes. NVP-DPP728 has been administered in a placebo-controlled, double-blind, multicentre study either at 100 mg t.i.d or 150 mg orally b.i.d. for 4 weeks to 93 patients with diet-controlled type 2 diabetes; mean age 64, prior duration of diabetes ~ 3.6 years, body-mass index (BMI) 27.2, with a mean fasting glucose of 8.5 and a HbA1c of 7.4% prior to drug treatment [158]. Both treatment regimens significantly improved mean 24-h glucose excursion with a reduction in mean 24-h insulin levels noted in treated subjects. Fasting and postprandial plasma glucose was also significantly reduced in both treatment arms, as was HbA1c. Body weight was not changed during the 4 week study period. Four drug-treated patients experienced symptoms compatible with nasopharyngitis and five patients complained of pruritus primarily localised to the palms. However, these symptoms were transient, with pruritus disappearing within 48 h without need for discontinuation of therapy. One patient with pre-existing albuminuria developed transient

nephrotic syndrome during the first week of treatment, leading to discontinuation of therapy.

The efficacy of the orally available inhibitor P32/98 has also been examined in both healthy normal subjects and in patients with type 2 diabetes [159]. A single 60 mg oral dose produced a rapid inhibition of plasma DPP-IV activity within 45 min of drug administration. When P32/98 was given 15 min prior to an oral glucose tolerance test in healthy volunteers, increased levels of bioactive intact GLP-1 were detected in drug-treated subjects. Analysis of the effects of single dose P32/98 on glucose excursion in diabetic subjects revealed reduced glucose area under the curve for patients previously treated with acarbose and glibenclamide [159]. The effects of long-term treatment with P32/98 in diabetic subjects have not yet been reported.

4. Expert opinion

The important observations that both GLP-1 and GIP are rapidly cleaved at the N-terminus, followed by the identification of DPP-IV as an essential determinant of incretin inactivation, has fostered considerable interest in evaluation of DPP-IV inhibitor therapy for the treatment of type 2 diabetes. Concurrently, multiple long-acting GLP-1R agonists are being evaluated in the clinic in Phase II/III trials in diabetic subjects. The theoretical advantages of GLP-1R agonists (Table 2) include the ability to achieve much greater and sustained levels of circulating bioactive GLP-1, which should provide more robust and sustained activation of GLP-1Rs coupled to glucose lowering. Furthermore, injectable GLP-1 analogues are likely to be more potent inducers of satiety and inhibitors of gastric emptying, and they have been shown to regulate islet cell proliferation and cytoprotection. In contrast, although DPP-IV inhibitors are orally available and potentially more attractive to patients, they are less well characterised with respect to their spectrum of incretin-like actions and safety profile (Table 2) and are predicted to be less potent than injectable GLP-1 analogues in the acute lowering of plasma glucose.

Hence, several important questions and challenges remain if this class of agents is to be developed successfully and safely for use in the diabetes clinic. The large number of potential DPP-IV substrates, encompassing gut and CNS regulatory peptides, chemokines and vasoactive peptides, suggests that predicting and understanding the biology of transient or sustained DPP-IV inhibition in human subjects may be difficult, even after exhaustive preclinical evaluation of highly specific compounds. Furthermore, the pleiotropic functions of DPP-IV, acting as both a membrane bound and soluble form and exerting diverse effects on lymphocyte signalling, cell migration and proliferation, at times independent of its enzymatic activity, provide further challenges for scientists seeking to understand how specific inhibition of enzymatic function may impact the non-enzymatic biological actions of DPP-IV in different human tissues. The relative long-term safety of compounds that produce transient versus more sustained DPP-IV inhibition cannot be inferred from available data, although studies of incretin biology and preclinical evaluation of DPP-IV inhibitors argue that continuous potentiation of incretin receptor signalling is likely to be more effective for the treatment of subjects with type 2 diabetes mellitus.

Table 2. Comparison of DPP-IV inhibitors versus GLP-1 analogues for the treatment of type 2 diabetes.

DPP-IV inhibitors	GLP-1 analogues
Orally available	Injectable
Multiple targets	Single known GPCR target
Stabilisation of endogenous GLP-1	Higher levels of circulating GLP-1 achievable
Short versus long acting	Longer acting; days to weeks?
Drug overdose non-toxic	Drug overdose potentially problematic
CNS side effects unlikely	Potential for CNS side effects
Potential for unanticipated toxicity	Biological actions more precisely defined

DPP-IV: Dipeptidyl peptidase IV; GLP-1: Glucagon-like peptide 1; GPCR: G-protein-coupled receptor.

Although the pharmaceutical industry is developing multiple potent, highly specific, DPP-IV inhibitor compounds with favourable pharmacokinetic profiles, the biology and consequences of sustained DPP-IV inhibition may be different in comparatively well patients with type 2 diabetes versus more complex older diabetic subjects with additional coexisting illnesses. For example, the effects and putative safety of chronic DPP-IV inhibitor therapy in diabetic patients with coexistent immune or inflammatory disorders, atopy, angioedema or malignancy, cannot be inferred with any degree of confidence from preclinical or short-term clinical studies. Nevertheless, despite these concerns, the surprising potency of these compounds in experimental models of type 2 diabetes, the need for new effective medications to treat type 2 diabetes, taken together with the preliminary data demonstrating efficacy in short-term clinical trials, argues for ongoing assessment and evaluation of these compounds as new therapeutic agents. More selective approaches for targeting the DPP-IV enzyme, for example with tissue-specific inhibitors, are under development and may offer theoretical advantages for restricting drug activity to one or more localised tissue compartments. Similarly, DPP-IV inhibitors have been proposed as agents for the treatment of immune or CNS disorders. However, insufficient data are available to provide informed opinion about the scientific merits of these strategies. As is the case for all new investigational agents representing innovative approaches to disease treatment, there will be no substitute for rigorous scientific assessment of the specificity, mechanisms of action, safety and efficacy for each new DPP-IV compound that enters clinical development for the treatment of type 2 diabetes.

Bibliography

Papers of special note have been highlighted as of interest (•) to readers.

1. VILSBOLL T, KRARUP T, DEACON CF, MADSBAD S, HOLST JJ: Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in Type 2 diabetic patients. *Diabetes* (2001) 50:609-613.
• **This paper describes the extent of GLP-1 deficiency in human subjects with diabetes.**
2. RASK E, OLSSON T, SODERBERG S *et al.*: Impaired incretin response after a mixed meal is associated with insulin resistance in nondiabetic men. *Diabetes Care* (2001) 24:1640-1645.
3. DUPRE J, ROSS SA, WATSON D, BROWN JC: Stimulation of insulin secretion by gastric inhibitory polypeptide in man. *J. Clin. Endocrinol. Metab.* (1973) 37:826-828.
4. MEIER JJ, NAUCK MA, SCHMIDT WE, GALLWITZ B: Gastric Inhibitory Polypeptide: the neglected incretin revisited. *Regul. Pept.* (2002) 107:1-13.
5. BAGGIO L, KIEFFER TJ, DRUCKER DJ: GLP-1 but not GIP regulates fasting and non-enteral glucose clearance in mice. *Endocrinology* (2000) 141:3703-3709.
6. LEWIS JT, DAYANANDAN B, HABENER JF, KIEFFER TJ: Glucose-dependent insulinotropic polypeptide confers early phase insulin release to oral glucose in rats: demonstration by a receptor antagonist. *Endocrinology* (2000) 141:3710-3716.
7. MIYAWAKI K, YAMADA Y, YANO H *et al.*: Glucose intolerance caused by a defect in the entero-insular axis: A study in gastric inhibitory polypeptide receptor knockout mice. *Proc. Natl. Acad. Sci. USA* (1999) 96:14843-14847.
8. YIP RG, WOLFE MM: GIP biology and fat metabolism. *Life Sci.* (2000) 66:91-103.
9. MIYAWAKI K, YAMADA Y, BAN N *et al.*: Inhibition of gastric inhibitory polypeptide signaling prevents obesity. *Nat. Med.* (2002) 8:738-742.
10. LYNN FC, PAMIR N, NG EH, MCINTOSH CH, KIEFFER TJ, PEDERSON RA: Defective glucose-dependent insulinotropic polypeptide receptor expression in diabetic fatty Zucker rats. *Diabetes* (2001) 50:1004-1011.
11. JONES IR, OWENS DR, MOODY AJ, LUZIO SD, MORRIS T, HAYES TM: The effects of glucose-dependent insulinotropic polypeptide infused at physiological concentrations in normal subjects and Type 2 (non-insulin- dependent) diabetic patients on glucose tolerance and B-cell secretion. *Diabetologia* (1987) 30:707-712.
12. NAUCK MA, HEIMESAAT MM, ORSKOV C, HOLST JJ, EBERT R, CREUTZFELDT W: Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J. Clin. Invest.* (1993) 91:301-307.
13. ELAHI D, MCALOON-DYKE M, FUKAGAWA NK *et al.*:The insulinotropic actions of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (7-37) in normal and diabetic subjects. *Regul. Pept.* (1994) 51:63-74.
14. VILSBOLL T, KRARUP T, MADSBAD S, HOLST JJ: Defective amplification of the late phase insulin response to glucose by GIP in obese Type II diabetic patients. *Diabetologia* (2002) 45:1111-1119.
15. MEIER JJ, HUCKING K, HOLST JJ, DEACON CF, SCHMIEGEL WH, NAUCK MA: Reduced insulinotropic effect of gastric inhibitory polypeptide in first-degree relatives of patients with type 2 diabetes. *Diabetes* (2001) 50:2497-2504.
16. O'HARTE FP, GAULT VA, PARKER JC *et al.*: Improved stability, insulin-releasing activity and antidiabetic potential of two novel N-terminal analogues of gastric inhibitory polypeptide: N-acetyl-GIP and pGlu-GIP. *Diabetologia* (2002) 45:1281-1291.
17. LUGARI R, DEI CAS A, UGOLOTTI D *et al.*: Evidence for early impairment of glucagon-like peptide 1-induced insulin secretion in human Type 2 (non insulin-dependent) diabetes. *Horm. Metab. Res.* (2002) 34:150-154.
18. RUIZ-GRANDE C, PINTADO J, ALARCON C, CASTILLA C, VALVERDE I, LOPEZ-NOVOA JM: Renal catabolism of human glucagon-like peptides 1 and 2. *Can. J. Physiol. Pharmacol.* (1990) 68:1568-1573.
19. RUIZ-GRANDE C, ALARCON C, ALCANTARA A *et al.*: Renal catabolism of truncated glucagon-like peptide 1. *Horm. Metab. Res.* (1993) 25:612-616.
20. MENTLEIN R, GALLWITZ B, SCHMIDT WE: Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur. J. Biochem.* (1993) 214:829-835.
• **An early original description of the importance of DPP-IV for N-terminal inactivation of incretin hormones.**
21. KIEFFER TJ, MCINTOSH CH, PEDERSON RA: Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 *in vitro* and *in vivo* by dipeptidyl peptidase IV. *Endocrinology* (1995) 136:3585-3596.
22. DEACON CF, NAUCK MA, TOFT-NIELSEN M, PRIDAL L, WILLMS B, HOLST JJ: Both subcutaneously and intravenously administered glucagon-like peptide 1 are rapidly degraded from the NH2-terminus in Type II diabetic patients and in healthy subjects. *Diabetes* (1995) 44:1126-1131.
23. HUPE-SODMANN K, MCGREGOR GP, BRIDENBAUGH R *et al.*: Characterisation of the processing by human neutral endopeptidase 24.11 of GLP-1(7-36) amide and comparison of the substrate specificity of the enzyme for other glucagon-like peptides. *Regul. Pept.* (1995) 58:149-156.
24. DRUCKER DJ, PHILIPPE J, MOJSOV S, CHICK WL, HABENER JF: Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc. Natl. Acad. Sci. USA* (1987) 84:3434-3438.
25. MOJSOV S, WEIR GC, HABENER JF: Insulinotropin: Glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J. Clin. Invest.* (1987) 79:616-619.
26. ORSKOV C, HOLST JJ, NIELSEN OV: Effect of truncated glucagon-like peptide-1 [proglucagon-(78-107) amide] on endocrine secretion from pig pancreas, antrum, and nonantral stomach. *Endocrinology* (1988) 123:2009-2013.
27. HOLZ GG, KUHTREIBER WM, HABENER JF: Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7-37). *Nature* (1993) 361:362-365.
• **Experiments describing the effects of GLP-1 on recruitment of glucose-competent β cells, with implications for GLP-1-dependent enhancement of human β cell activity.**

28. WANG Y, EGAN JM, RAYGADA M, NADIV O, ROTH J, MONTROSE-RAFIZADEH C: Glucagon-like peptide-1 affects gene transcription and messenger ribonucleic acid stability of components of the insulin secretory system in RIN 1046-38 cells. *Endocrinology.* (1995) 136(11):4910-4917.

29. FEHMANN H-C, HABENER JF: Insulinotropic hormone glucagon-like peptide-I(7-37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma βTC-1 cells. *Endocrinology* (1992) 130:159-166.

30. WANG Y, PERFETTI R, GREIG NH *et al.*: Glucagon-like peptide-1 can reverse the age-related decline in glucose tolerance in rats. *J. Clin. Invest.* (1997) 99:2883-2889.

31. WILLMS B, WERNER J, HOLST JJ, ORSKOV C, CREUTZFELDT W, NAUCK MA: Gastric emptying, glucose responses, and insulin secretion after a liquid test meal: effects of exogenous glucagon-like peptide-1 (GLP-1)-(7-36)amide in Type 2 (non-insulin dependent) diabetic patients. *J. Clin. Endocrinol. Metab.* (1996) 81:327-332.

32. NAUCK MA, NIEDEREICHHOLZ U, ETTLER R *et al.*: Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. *Am. J. Physiol.* (1997) 273:E981-E988.

33. KAWAI K, SUZUKI S, OHASHI S, MUKAI H, MURAYMA Y, YAMASHITA K: Effects of truncated glucagon-like peptide-1 on pancreatic hormone release in normal conscious dogs. *Acta Endocrinol. (Copenh.)* (1990) 123:661-667.

34. KASHIMA Y, MIKI T, SHIBASAKI T *et al.*: Critical role of cAMP-GEFII/Rim2 complex in incretin-potentiated insulin secretion. *J. Biol. Chem.* (2001) 276:46046-46053.

35. HOLZ GG, LEECH CA, HABENER JF: Activation of a cAMP-regulated Ca^{2+}-signaling pathway in pancreatic b-cells by the insulinotropic hormone glucagon-like peptide-1. *J. Biol. Chem.* (1995) 270:17749-17757.

36. LIGHT PE, MANNING FOX JE, RIEDEL MJ, WHEELER MB: Glucagon-like peptide-1 inhibits pancreatic ATP-sensitive potassium channels via a protein kinase A- and ADP-dependent mechanism. *Mol. Endocrinol.* (2002) 16:2135-2144.

37. EDVELL A, LINDSTROM P: Initiation of increased pancreatic islet growth in young normoglycemic mice (Umea +/?). *Endocrinology* (1999) 140:778-783.

38. XU G, STOFFERS DA, HABENER JF, BONNER-WEIR S: Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes* (1999) 48:2270-2276.

39. STOFFERS DA, KIEFFER TJ, HUSSAIN MA *et al.*: Insulinotropic glucagon-like peptide-1 agonists stimulate expression of homeodomain protein IDX-1 and increase β-cell mass in mouse pancreas. *Diabetes* (2000) 49:741-748.

40. ZHOU J, WANG X, PINEYRO MA, EGAN JM: Glucagon-like peptide 1 and exendin-4 convert pancreatic AR42J cells into glucagon- and insulin-producing cells. *Diabetes* (1999) 48:2358-2366.

41. HUI H, WRIGHT C, PERFETTI R: Glucagon-like peptide 1 induces differentiation of islet duodenal homeobox-1-positive pancreatic ductal cells into insulin-secreting cells. *Diabetes* (2001) 50:785-796.

42. DRUCKER DJ: Glucagon-like peptides: Regulators of cell proliferation, differentiation and apoptosis. *Mol. Endocrinol.* (2003) 17 (In press).

43. BEAK SA, HEATH MM, SMALL CJ *et al.*: Glucagon-like peptide-1 stimulates luteinizing hormone releasing hormone secretion in a rodent hypothalamic neuronal cell line (GT1-7). *J. Clin. Invest.* (1998) 101:1334-1341.

44. BEAK SA, SMALL CJ, ILOVAISKAIA I *et al.*: Glucagon-like peptide-1 (GLP-1) releases thyrotropin (TSH): characterization of binding sites for GLP-1 on alpha-TSH cells. *Endocrinology* (1996) 137:4130-4138.

45. LARSEN PJ, TANG-CHRISTENSEN M, JESSOP DS: Central administration of glucagon-like peptide-1 activates hypothalamic neuroendocrine neurons in the rat. *Endocrinology* (1997) 138:4445-4455.

46. TURTON MD, O'SHEA D, GUNN I *et al.*: A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* (1996) 379:69-72.

- **Original observation describing potent satiety effects following intracerebroventricular GLP-1 administration.**

47. DONAHEY JCK, VAN DIJK G, WOODS SC, SEELEY RJ: Intraventricular GLP-1 reduces short- but not long-term food intake or body weight in lean and obese rats. *Brain Res.* (1998) 779:75-83.

48. YOUNG AA, GEDULIN BR, BHAVSAR S *et al.*: Glucose-lowering and insulin-sensitizing actions of exendin-4: studies in obese diabetic (ob/ob, db/db) mice, diabetic fatty Zucker rats, and diabetic rhesus monkeys (Macaca mulatta). *Diabetes* (1999) 48:1026-1034.

49. LARSEN PJ, FLEDELIUS C, KNUDSEN LB, TANG-CHRISTENSEN M: Systemic administration of the long-acting GLP-1 derivative NN2211 induces lasting and reversible weight loss in both normal and obese rats. *Diabetes* (2001) 50:2530-2539.

50. VERDICH C, FLINT A, GUTZWILLER JP *et al.*: A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on ad libitum energy intake in humans. *J. Clin. Endocrinol. Metab.* (2001) 86:4382-4389.

51. ZANDER M, MADSBAD S, MADSEN JL, HOLST JJ: Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in Type 2 diabetes: a parallel-group study. *Lancet* (2002) 359:824-830.

- **Long-term 6-week GLP-1 infusion study in human diabetics demonstrating improvement in multiple metabolic parameters and significant lowering of HbA1c.**

52. WANG Z, WANG RM, OWJI AA, SMITH DM, GHATEI MA, BLOOM SR: Glucagon-like peptide 1 is a physiological incretin in rat. *J. Clin. Invest.* (1995) 95:417-421.

53. KOLLIGS F, FEHMANN H-C, GOKE R, GOKE B: Reduction of the incretin effect in rats by the glucagon-like peptide 1 receptor antagonist exendin (9-39) amide. *Diabetes* (1995) 44:16-19.

54. EDWARDS CM, TODD JF, MAHMOUDI M *et al.*: Glucagon-like peptide 1 has a physiological role in the control of postprandial glucose in humans: studies with the antagonist exendin 9-39. *Diabetes* (1999) 48:86-93.

55. SCHIRRA J, STURM K, LEICHT P, ARNOLD R, GOKE B, KATSCHINSKI M: Exendin(9-39)amide is an antagonist of glucagon-like peptide-1(7- 36)amide in humans. *J. Clin. Invest.* (1998) 101:1421-1430.

56. SCROCCHI LA, BROWN TJ, MACLUSKY N *et al.*: Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide

receptor gene. *Nat. Med.* (1996) 2:1254-1258.

- **Genetic inactivation of the GLP-1 receptor demonstrates the physiological importance of GLP-1 for glucose control in mice.**

57. MEERAN K, O'SHEA D, EDWARDS CM *et al.*: Repeated intracerebroventricular administration of glucagon-like peptide-1-(7-36) amide or exendin-(9-39) alters body weight in the rat. *Endocrinology* (1999) 140:244-250.
58. SCROCCHI LA, DRUCKER DJ: Effects of aging and a high fat diet on body weight and glucose control in GLP-1R-/- mice. *Endocrinology* (1998) 139:3127-3132.
59. LING Z, WU D, ZAMBRE Y *et al.*: Glucagon-like peptide 1 receptor signaling influences topography of islet cells in mice. *Virchows Arch.* (2001) 438:382-387.
60. SCROCCHI LA, HILL ME, SALEH J, PERKINS B, DRUCKER DJ: Elimination of GLP-1R signaling does not modify weight gain and islet adaptation in mice with combined disruption of leptin and GLP-1 action. *Diabetes* (2000) 49:1552-1560.
61. PEDERSON RA, SATKUNARAJAH M, MCINTOSH CH *et al.*: Enhanced glucose-dependent insulinotropic polypeptide secretion and insulinotropic action in glucagon-like peptide 1 receptor -/- mice. *Diabetes* (1998) 47:1046-1052.
62. GUTNIAK M, ORSKOV C, HOLST JJ, AHREN B, EFENDIC S: Antidiabetogenic effect of glucagon-like peptide-1 (7-36)amide in normal subjects and patients with diabetes mellitus. *N. Engl. J. Med.* (1992) 326:1316-1322.
63. EDWARDS CM, STANLEY SA, DAVIS R *et al.*: Exendin-4 reduces fasting and postprandial glucose and decreases energy intake in healthy volunteers. *Am. J. Physiol. Endocrinol. Metab.* (2001) 281:E155-E161.
64. GUTNIAK MK, LINDE B, HOLST JJ, EFENDIC S: Subcutaneous injection of the incretin hormone glucagon-like peptide 1 abolishes postprandial glycemia in NIDDM. *Diabetes Care* (1994) 17:1039-1044.
65. RACHMAN J, GRIBBLE FM, BARROW BA, LEVY JC, BUCHANAN KD, TURNER RC: Normalization of insulin responses to glucose by overnight infusion of glucagon-like peptide 1(7-36)amide in patients with NIDDM. *Diabetes* (1996) 45:1524-1530.
66. CREUTZFELD WO, KLEINE N, WILLMS B, ORSKOV C, HOLST JJ, NAUCK MA: Glucagonostatic actions and reduction of fasting hyperglycemia by exogenous glucagon-like peptide I(7-36) amide in Type I diabetic patients. *Diabetes Care* (1996) 19:580-586.
67. NATHAN DM, SCHREIBER E, FOGEL H, MOJSOV S, HABENER JF: Insulinotropic action of glucagon-like peptide-I-(7-37) in diabetic and non-diabetic subjects. *Diabetes Care* (1992) 15:270-276.
68. GUTNIAK MK, JUNTTI-BERGGREN L, HELLSTROM PM, GUENIFI A, HOLST JJ, EFENDIC S: Glucagon-like peptide I enhances the insulinotropic effect of glibenclamide in NIDDM patients and in the perfused rat pancreas. *Diabetes Care* (1996) 19:857-863.
69. DUPRE J, BEHME MT, HRAMIAK IM *et al.*: Glucagon-like peptide I reduces postprandial glycemic excursions in IDDM. *Diabetes* (1995) 44:626-630.
70. DEACON CF, JOHNSEN AH, HOLST JJ: Degradation of glucagon-like peptide-1 by human plasma *in vitro* yields an N-terminally truncated peptide that is a major endogenous metabolite *in vivo*. *J. Clin. Endocrinol. Metab.* (1995) 80:952-957.
71. ORSKOV C, WETTERGREN A, HOLST JJ: Biological effects and metabolic rates of glucagon-like peptide-1 7-36 amide and glucagonlike peptide-1 7-37 in healthy subjects are indistinguishable. *Diabetes* (1993) 42:658-661.
72. LARSEN J, HYLLEBERG B, NG K, DAMSBO P: Glucagon-like peptide-1 infusion must be maintained for 24 h/day to obtain acceptable glycemia in type 2 diabetic patients who are poorly controlled on sulphonylurea treatment. *Diabetes Care* (2001) 24:1416-1421.
73. DRUCKER DJ: Development of glucagon-like peptide-1-based pharmaceuticals as therapeutic agents for the treatment of diabetes. *Curr. Pharm. Des.* (2001) 7:1399-1412.
74. RACHMAN J, BARROW BA, LEVY JC, TURNER RC: Near normalization of diurnal glucose concentrations by continuous administration of glucagon-like peptide 1 (GLP-1) in subjects with NIDDM. *Diabetologia* (1997) 40:205-211.
75. TOFT-NIELSEN MB, MADSBAD S, HOLST JJ: Continuous subcutaneous infusion of glucagon-like peptide 1 lowers plasma glucose and reduces appetite in Type 2 diabetic patients. *Diabetes Care* (1999) 22:1137-1143.
76. TODD JF, EDWARDS CM, GHATEI MA, MATHER HM, BLOOM SR: Subcutaneous glucagon-like peptide-1 improves postprandial glycaemic control over a 3-week period in patients with early Type 2 diabetes. *Clin. Sci. (Colch.)* (1998) 95:325-329.
77. GOKE R, FEHMANN H-C, LINN T *et al.*: Exendin-4 is a high potency agonist and truncated exendin-(9-39)-amide an antagonist at the glucagon-like peptide 1-(7-36)-amide receptor of insulin-secreting β-cells. *J. Biol. Chem.* (1993) 268:19650-19655.
78. EGAN JM, CLOCQUET AR, ELAHI D: The insulinotropic effect of acute exendin-4 administered to humans: comparison of nondiabetic state to Type 2 diabetes. *J. Clin. Endocrinol. Metab.* (2002) 87:1282-1290.
79. AGERSO H, JENSEN LB, ELBROND B, ROLAN P, ZDRAVKOVIC M: The pharmacokinetics, pharmacodynamics, safety and tolerability of NN2211, a new long-acting GLP-1 derivative, in healthy men. *Diabetologia* (2002) 45:195-202.
80. JUHL CB, HOLLINGDAL M, STURIS J: Bedtime administration of NN2211, a long-acting GLP-1 derivative, substantially reduces fasting and postprandial glycemia in Type 2 diabetes. *Diabetes* (2002) 51:424-429.
81. MENTLEIN R: Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. *Regul. Pept.* (1999) 85:9-24.
82. GORRELL MD, GYSBERS V, MCCAUGHAN GW: CD26: a multifunctional integral membrane and secreted protein of activated lymphocytes. *Scand. J. Immunol.* (2001) 54:249-264.
83. IWAKI-EGAWA S, WATANABE Y, KIKUYA Y, FUJIMOTO Y: Dipeptidyl peptidase IV from human serum: purification, characterization, and N-terminal amino acid sequence. *J. Biochem. (Tokyo)* (1998) 124:428-433.
84. DURINX C, LAMBEIR AM, BOSMANS E *et al.*: Molecular characterization of dipeptidyl peptidase activity in serum: soluble CD26/dipeptidyl peptidase IV is responsible for the release of X-Pro dipeptides. *Eur. J. Biochem.* (2000) 267:5608-5613.
85. DAVID F, BERNARD AM, PIERRES M, MARGUET D: Identification of serine

624, aspartic acid 702, and histidine 734 as the catalytic triad residues of mouse dipeptidyl-peptidase IV (CD26). A member of a novel family of nonclassical serine hydrolases. *J. Biol. Chem* (1993) 268:17247-17252.

86. ABBOTT CA, BAKER E, SUTHERLAND GR, MCCAUGHAN GW: Genomic organization, exact localization, and tissue expression of the human CD26 (dipeptidyl peptidase IV) gene. *Immunogenetics* (1994) 40:331-338.

87. HONG WJ, PETELL JK, SWANK D, SANFORD J, HIXSON DC, DOYLE D: Expression of dipeptidyl peptidase IV in rat tissues is mainly regulated at the mRNA levels. *Exp. Cell Res.* (1989) 182:256-266.

88. SAKAMOTO J, WATANABE T, TERAMUKAI S *et al.*: Distribution of adenosine deaminase binding protein in normal and malignant tissues of the gastrointestinal tract studied by monoclonal antibodies. *J. Surg. Oncol.* (1993) 52:124-134.

89. MORIMOTO C, SCHLOSSMAN SF: The structure and function of CD26 in the T-cell immune response. *Immunol. Rev.* (1998) 161:55-70.

90. HILDEBRANDT M, REUTTER W, ARCK P, ROSE M, KLAPP BF: A guardian angel: the involvement of dipeptidyl peptidase IV in psychoneuroendocrine function, nutrition and immune defence. *Clin. Sci. (Lond.)* (2000) 99:93-104.

91. OHTSUKI T, TSUDA H, MORIMOTO C: Good or evil: CD26 and HIV infection. *J. Dermatol. Sci.* (2000) 22:152-160.

92. IWATA S, MORIMOTO C: CD26/dipeptidyl peptidase IV in context. The different roles of a multifunctional ectoenzyme in malignant transformation. *J. Exp. Med.* (1999) 190:301-306.

93. DANG NH, MORIMOTO C: CD26: an expanding role in immune regulation and cancer. *Histol. Histopathol.* (2002) 17:1213-1226.

94. ISHII T, OHNUMA K, MURAKAMI A *et al.*: CD26-mediated signaling for T cell activation occurs in lipid rafts through its association with CD45RO. *Proc. Natl. Acad. Sci. USA* (2001) 98:12138-12143.

95. BUHLING F, KUNZ D, REINHOLD D *et al.*: Expression and functional role of dipeptidyl peptidase IV (CD26) on human natural killer cells. *Nat. Immun.* (1994) 13:270-279.

96. HEGEN M, KAMEOKA J, DONG RP, MORIMOTO C, SCHLOSSMAN SF: Structure of CD26 (dipeptidyl peptidase IV) and function in human T cell activation. *Adv. Exp. Med. Biol.* (1997) 421:109-116.

97. HEGEN M, KAMEOKA J, DONG RP, SCHLOSSMAN SF, MORIMOTO C: Cross-linking of CD26 by antibody induces tyrosine phosphorylation and activation of mitogen-activated protein kinase. *Immunology* (1997) 90:257-264.

98. IKUSHIMA H, MUNAKATA Y, ISHII T *et al.*: Internalization of CD26 by mannose 6-phosphate/insulin-like growth Factor II receptor contributes to T cell activation. *Proc. Natl. Acad. Sci. USA* (2000) 97:8439-8444.

99. IKUSHIMA H, MUNAKATA Y, IWATA S *et al.*: Soluble CD26/dipeptidyl peptidase IV enhances transendothelial migration via its interaction with mannose 6-phosphate/insulin-like growth Factor II receptor. *Cell Immunol.* (2002) 215:106-110.

100. OHNUMA K, MUNAKATA Y, ISHII T *et al.*: Soluble CD26/dipeptidyl peptidase IV induces T cell proliferation through CD86 up-regulation on APCs. *J. Immunol.* (2001) 167:6745-6755.

101. OHNUMA K, ISHII T, IWATA S *et al.*: G1/S cell cycle arrest provoked in human T cells by antibody to CD26. *Immunology* (2002) 107:325-333.

102. COBURN MC, HIXSON DC, REICHNER JS: *In vitro* immune responsiveness of rats lacking active dipeptidylpeptidase IV. *Cell Immunol.* (1994) 158:269-280.

103. MARGUET D, BAGGIO L, KOBAYASHI T *et al.*: Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proc. Natl. Acad. Sci. USA* (2000) 97:6874-6879.

• **Mice deficient in CD26 exhibit increased levels of circulating incretins, reduced blood glucose and increased levels of plasma insulin following nutrient challenge.**

104. PERNER F, GYURIS T, RAKOCZY G *et al.*: Dipeptidyl peptidase activity of CD26 in serum and urine as a marker of cholestasis: experimental and clinical evidence. *J. Lab. Clin. Med.* (1999) 134:56-67.

105. FIRNEISZ G, LAKATOS PL, SZALAY F: Serum dipeptidyl peptidase IV (DPP IV, CD26) activity in chronic hepatitis C. *Scand. J. Gastroenterol.* (2001) 36:877-880.

106. GOTOH H, HAGIHARA M, NAGATSU T, IWATA H, MIURA T: Activity of dipeptidyl peptidase IV and post-proline cleaving enzyme in sera from osteoporotic patients. *Clin. Chem.* (1988) 34:2499-2501.

107. REINHOLD D, HEMMER B, GRAN B *et al.*: Dipeptidyl peptidase IV (CD26): role in T cell activation and autoimmune disease. *Adv. Exp. Med. Biol.* (2000) 477:155-160.

108. VANHAM G, KESTENS L, DE MEESTER I *et al.*: Decreased expression of the memory marker CD26 on both CD4+ and CD8+ T lymphocytes of HIV-infected subjects. *J. Acquir. Immune Defic. Syndr.* (1993) 6:749-757.

109. HOSONO O, HOMMAT, KOBAYASHI H *et al.*: Decreased dipeptidyl peptidase IV enzyme activity of plasma soluble CD26 and its inverse correlation with HIV-1 RNA in HIV-1 infected individuals. *Clin. Immunol.* (1999) 91:283-295.

110. KEANE NM, PRICE P, LEE S, STONE SF, FRENCH MA: An evaluation of serum soluble CD30 levels and serum CD26 (DPPIV) enzyme activity as markers of Type 2 and Type 1 cytokines in HIV patients receiving highly active antiretroviral therapy. *Clin. Exp. Immunol.* (2001) 126:111-116.

111. MENTLEIN R, STAVES R, RIX-MATZEN H, TINNEBERG HR: Influence of pregnancy on dipeptidyl peptidase IV activity (CD 26 leukocyte differentiation antigen) of circulating lymphocytes. *Eur. J. Clin. Chem. Clin. Biochem.* (1991) 29:477-480.

112. ROSE M, HILDEBRANDT M, FLIEGE H, SEIBOLD S, MONNIKES H, KLAPP BF: T-cell immune parameters and depression in patients with Crohn's disease. *J. Clin. Gastroenterol.* (2002) 34:40-48.

113. MAES M, DE MEESTER I, VERKERK R *et al.*: Lower serum dipeptidyl peptidase IV activity in treatment resistant major depression: relationships with immune-inflammatory markers. *Psychoneuroendocrinology* (1997) 22:65-78.

114. ELGUN S, KESKINEGE A, KUMBASAR H: Dipeptidyl peptidase IV and adenosine deaminase activity. Decrease in depression. *Psychoneuroendocrinology* (1999) 24:823-832.

115. HILDEBRANDTM, ROSE M, MAYR C *et al.*: Dipeptidyl peptidase IV (DPP IV, CD26) in patients with mental eating

disorders. *Adv. Exp. Med. Biol.* (2000) 477:197-204.

116. STANCIKOVA M, LOJDA Z, LUKAC J, RUZICKOVA M: Dipeptidyl peptidase IV in patients with systemic lupus erythematosus. *Clin. Exp. Rheumatol.* (1992) 10:381-385.
117. CORDERO OJ, SALGADO FJ, MERA-VARELA A, NOGUEIRA M: Serum interleukin-12, interleukin-15, soluble CD26, and adenosine deaminase in patients with rheumatoid arthritis. *Rheumatol. Int.* (2001) 21:69-74.
118. SCHONERMARCK U, CSERNOK E, TRABANDT A, HANSEN H, GROSS WL: Circulating cytokines and soluble CD23, CD26 and CD30 in ANCA-associated vasculitides. *Clin. Exp. Rheumatol.* (2000) 18:457-463.
119. LEFEBVRE J, MURPHEY LJ, HARTERT TV, JIAO SHAN R, SIMMONS WH, BROWN NJ: Dipeptidyl peptidase IV activity in patients with ACE-inhibitor-associated angioedema. *Hypertension* (2002) 39:460-464.
120. TANAKA T, UMEKI K, YAMAMOTO I, SAKAMOTO F, NOGUCHI S, OHTAKI S: CD26 (dipeptidyl peptidase IV/DPP IV) as a novel molecular marker for differentiated thyroid carcinoma. *Int. J. Cancer* (1995) 64:326-331.
121. UMEKI K, TANAKA T, YAMAMOTO I *et al.*: Differential expression of dipeptidyl peptidase IV (CD26) and thyroid peroxidase in neoplastic thyroid tissues. *Endocr. J.* (1996) 43:53-60.
122. WILSON MJ, RUHLAND AR, QUAST BJ, REDDY PK, EWING SL, SINHA AA: Dipeptidylpeptidase IV activities are elevated in prostate cancers and adjacent benign hyperplastic glands. *J. Androl.* (2000) 21:220-226.
123. CORDERO OJ, AYUDE D, NOGUEIRA M, RODRIGUEZ-BERROCAL FJ, DE LA CADENA MP: Preoperative serum CD26 levels: diagnostic efficiency and predictive value for colorectal cancer. *Br. J. Cancer* (2000) 83:1139-1146.
124. UEMATSU T, URADE M, YAMAOKA M, YOSHIOKA W: Reduced expression of dipeptidyl peptidase (DPP) IV in peripheral blood T lymphocytes of oral cancer patients. *J. Oral Pathol. Med.* (1996) 25:507-512.
125. LAMBEIR AM, PROOST P, DURINX C *et al.*: Kinetic investigation of chemokine truncation by CD26/dipeptidyl peptidase IV reveals a striking selectivity within the chemokine family. *J. Biol. Chem.* (2001) 276:29839-29845.
126. LAMBEIR AM, DURINX C, PROOST P, VAN DAMME J, SCHARPE S, DE MEESTER I: Kinetic study of the processing by dipeptidyl-peptidase IV/CD26 of neuropeptides involved in pancreatic insulin secretion. *FEBS Lett.* (2001) 507:327-330.
127. DE MEESTER I, DURINX C, BAL G *et al.*: Natural substrates of dipeptidyl peptidase IV. *Adv. Exp. Med. Biol.* (2000) 477:67-87.
128. POSPISILIK JA, HINKE SA, PEDERSON RA *et al.*: Metabolism of glucagon by dipeptidyl peptidase IV (CD26). *Regul. Pept.* (2001) 6:133-141.
129. HINKE SA, POSPISILIK JA, DEMUTH HU *et al.*: Dipeptidyl peptidase IV (DPIV/CD26) degradation of glucagon. Characterization of glucagon degradation products and DPIV-resistant analogs. *J. Biol. Chem.* (2000) 275:3827-3834.
130. POULSEN MD, HANSEN GH, DABELSTEEN E, HOYER PE, NOREN O, SJOSTROM H: Dipeptidyl peptidase IV is sorted to the secretory granules in pancreatic islet A-cells. *J. Histochem. Cytochem.* (1993) 41:81-88.
131. HANSEN L, DEACON CF, ORSKOV C, HOLST JJ: Glucagon-like peptide-1-(7-36)amide is transformed to glucagon-like peptide-1-(9-36)amide by dipeptidyl peptidase IV in the capillaries Suppl.ying the L cells of the porcine intestine. *Endocrinology* (1999) 140:5356-5363.
132. PAULY RP, DEMUTH HU, ROSCHE F *et al.*: Improved glucose tolerance in rats treated with the dipeptidyl peptidase IV (CD26) inhibitor Ile-thiazolidide. *Metabolism* (1999) 48:385-389.
133. BALKAN B, KWASNIK L, MISERENDINO R, HOLST JJ, LI X: Inhibition of dipeptidyl peptidase IV with NVP-DPP728 increases plasma GLP-1 (7-36 amide) concentrations and improves oral glucose tolerance in obese Zucker rats. *Diabetologia* (1999) 42:1324-1331.
134. DEACON CF, WAMBERG S, BIE P, HUGHES TE, HOLST JJ: Preservation of active incretin hormones by inhibition of dipeptidyl peptidase IV suppresses meal-induced incretin secretion in dogs. *J. Endocrinol.* (2002) 172:355-362.
135. REIMER MK, HOLST JJ, AHREN B *et al.*: Long-term inhibition of dipeptidyl peptidase IV improves glucose tolerance and preserves islet function in mice. *Eur. J. Endocrinol.* (2002) 146:717-727.
136. NAGAKURA T, YASUDA N, YAMAZAKI K *et al.*: Improved glucose tolerance via enhanced glucose-dependent insulin secretion in dipeptidyl peptidase IV-deficient Fischer rats. *Biochem. Biophys. Res. Commun.* (2001) 284:501-506.
137. KNUDSEN LB, PRIDAL L: Glucagon-like peptide-1-(9-36) amide is a major metabolite of glucagon-like peptide-1-(7-36)amide after *in vivo* administration to dogs and it acts as an antagonist on the pancreatic receptor. *Eur. J. Pharmacol.* (1996) 318:429-435.
138. AYTAC U, CLARET FX, HO L *et al.*: Expression of CD26 and its associated dipeptidyl peptidase IV enzyme activity enhances sensitivity to doxorubicin-induced cell cycle arrest at the G(2)/M checkpoint. *Cancer Res.* (2001) 61:7204-7210.
139. WESLEY UV, ALBINO AP, TIWARI S, HOUGHTON AN: A role for dipeptidyl peptidase IV in suppressing the malignant phenotype of melanocytic cells. *J. Exp. Med.* (1999) 190:311-322.
140. PETHIYAGODA CL, WELCH DR, FLEMING TP: Dipeptidyl peptidase IV (DPPIV) inhibits cellular invasion of melanoma cells. *Clin. Exp. Metastasis* (2000) 18:391-400.
141. KAJIYAMA H, KIKKAWA F, SUZUKI T, SHIBATA K, INO K, MIZUTANI S: Prolonged survival and decreased invasive activity attributable to dipeptidyl peptidase IV overexpression in ovarian carcinoma. *Cancer Res.* (2002) 62:2753-2757.
142. SATO Y, FUJIWARA H, HIGUCHI T *et al.*: Involvement of Dipeptidyl Peptidase IV in Extravillous Trophoblast Invasion and Differentiation. *J. Clin. Endocrinol. Metab.* (2002) 87:4287-4296.
143. GHERSI G, DONG H, GOLDSTEIN LA *et al.*: Regulation of fibroblast migration on collagenous matrix by a cell surface peptidase complex. *J. Biol. Chem.* (2002) 277(32):29231-29241.
144. STEINBRECHER A, REINHOLD D, QUIGLEY L *et al.*: Targeting dipeptidyl peptidase IV (CD26) suppresses autoimmune encephalomyelitis and up-regulates TGF-beta 1 secretion *in vivo*. *J. Immunol.* (2001) 166:2041-2048.
145. SHINOSAKI T, KOBAYASHI T, KIMURA K, KURIHARA H: Involvement of dipeptidyl peptidase IV in immune

complex-mediated glomerulonephritis. *Lab. Invest.* (2002) 82:505-513.

146. GROUZMANN E, MONOD M, LANDIS B *et al.*: Loss of dipeptidylpeptidase IV activity in chronic rhinosinusitis contributes to the neurogenic inflammation induced by substance P in the nasal mucosa. *FASEB J.* (2002) 16:1132-1134.
147. SEDO A, MALIK R: Dipeptidyl peptidase IV-like molecules: homologous proteins or homologous activities? *Biochim. Biophys. Acta* (2001) 1550:107-116.
148. HOLST JJ, DEACON CF: Inhibition of the activity of dipeptidyl-peptidase IV as a treatment for Type 2 diabetes. *Diabetes* (1998) 47:1663-1670.
149. DEACON CF, HOLST JJ: Dipeptidyl peptidase IV inhibition as an approach to the treatment and prevention of Type 2 diabetes: a historical perspective. *Biochem. Biophys. Res. Commun.* (2002) 294:1-4.
150. DEMUTH HU, HINKE SA, PEDERSON RA, MCINTOSH CH: Rebuttal to Deacon and Holst: 'Metformin effects on dipeptidyl peptidase IV degradation of glucagon-like peptide-1' versus 'Dipeptidyl peptidase inhibition as an approach to the treatment and prevention of Type 2 diabetes: a historical perspective'. *Biochem. Biophys. Res. Commun.* (2002) 296:229-232.
151. DEACON CF, HUGHES TE, HOLST JJ: Dipeptidyl peptidase IV inhibition potentiates the insulinotropic effect of glucagon-like peptide 1 in the anesthetized pig. *Diabetes* (1998) 47:764-769.
152. PEDERSON RA, WHITE HA, SCHLENZIG D, PAULY RP, MCINTOSH CH, DEMUTH HU: Improved glucose tolerance in Zucker fatty rats by oral administration of the dipeptidyl peptidase IV inhibitor isoleucine thiazolidide. *Diabetes* (1998) 47:1253-1258.
153. AHREN B, HOLST JJ, MARTENSSON H, BALKAN B: Improved glucose tolerance and insulin secretion by inhibition of dipeptidyl peptidase IV in mice. *Eur. J. Pharmacol.* (2000) 404:239-245.
154. DEACON CF, DANIELSEN P, KLARSKOV L, OLESEN M, HOLST JJ: Dipeptidyl peptidase IV inhibition reduces the degradation and clearance of GIP and potentiates its insulinotropic and antihyperglycemic effects in anesthetized pigs. *Diabetes* (2001) 50:1588-1597.
155. POSPISILIK JA, STAFFORD SG, DEMUTH HU *et al.*: Long-term treatment with the dipeptidyl peptidase IV inhibitor P32/98 causes sustained improvements in glucose tolerance, insulin sensitivity, hyperinsulinemia, and beta-cell glucose responsiveness in VDF (fa/fa) Zucker rats. *Diabetes* (2002) 51:943-950.
156. POSPISILIK JA, STAFFORD SG, DEMUTH HU, MCINTOSH CH, PEDERSON RA: Long-term treatment with dipeptidyl peptidase iv inhibitor improves hepatic and peripheral insulin sensitivity in the VDF Zucker rat: a euglycemic-hyperinsulinemic clamp study. *Diabetes* (2002) 51:2677-2683.
157. SUDRE B, BROQUA P, WHITE RB *et al.*: Chronic inhibition of circulating dipeptidyl peptidase IV by FE 999011 delays the occurrence of diabetes in male zucker diabetic fatty rats. *Diabetes* (2002) 51:1461-1469.
158. AHREN B, SIMONSSON E, LARSSON H *et al.*: Inhibition of dipeptidyl peptidase IV improves metabolic control over a 4-week study period in Type 2 diabetes. *Diabetes Care* (2002) 25:869-875.
   - **Demonstration that a 4-week treatment course with a DPP-IV inhibitor lowers blood glucose and HbA1c in human diabetic subjects.**
159. HOFFMANN T, GLUND K, MCINTOSH CHS *et al.*:. DPPIV inhibition as treatment of type II diabetes. In: *Cell-Surface Aminopeptidases: Basic and Clinical Aspects.* S Mizutani (Ed.), Elsevier (2001):381-387.

Affiliation

DJ Drucker
Banting and Best Diabetes Centre, Toronto General Hospital, 200 Elizabeth Street MBRW4R-902, Toronto, Ontario, M5G 2C4, Canada
Tel: +416 340 4125; Fax: +416 978 4108;
E-mail: d.drucker@utoronto.ca

Dipeptidyl Peptidase IV Inhibitors for the Treatment of Diabetes

Ann E. Weber†

Merck Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065

Received December 24, 2003

Introduction

Ingestion of food results in the release of peptide hormones in the gut, termed incretins, that regulate insulin in a glucose-dependent manner.[1] When blood glucose levels are high, the incretin hormone glucagon-like peptide 1 (GLP-1[7–36] amide or GLP-1) stimulates insulin secretion and biosynthesis and inhibits glucagon release. In addition, it serves as an "ileal brake", slowing gastric emptying and reducing appetite. GLP-1 also appears to regulate the growth and differentiation of the insulin-producing β cells in pancreatic islets in rodents. Thus, GLP-1 therapy for the treatment of type 2 diabetes is an area of active research.[2]

GLP-1 is rapidly degraded in vivo through the action of dipeptidyl peptidase IV (DPP-IV), which cleaves the N-terminal two amino acids to give the inactive GLP-1[9–36] amide (Figure 1).[3] Thus, GLP-1 must be administered via chronic infusion in order to achieve sustained elevated plasma levels. DPP-IV resistant GLP-1 analogues represent one means to circumvent this issue, but like GLP-1, these are peptides that must be administered parenterally. Orally bioavailable, small-molecule agonists of the GLP-1 receptor have yet to be reported, though several patents claim low molecular weight GLP-1 agonists and potentiators.[4] Inhibition of DPP-IV, which leads to an increase in circulating levels of endogenous GLP-1, is an alternative approach that appears highly amenable to drug discovery.[5]

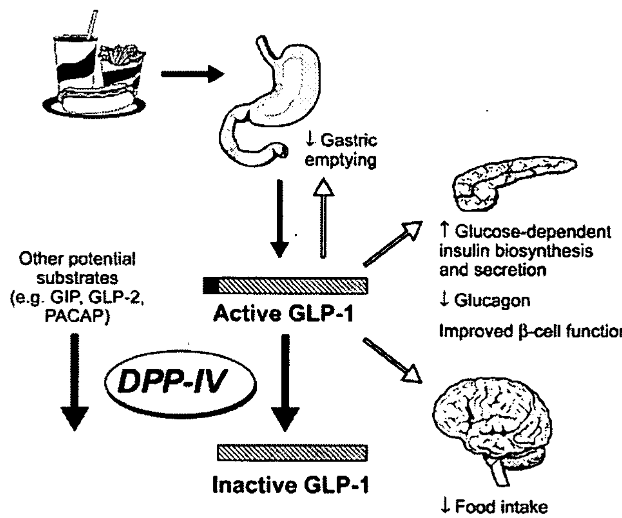


Figure 1. DPP-IV regulates glucose homeostasis via inactivation of GLP-1 and other incretin hormones.

DPP-IV Substrates

A cell surface serine protease, DPP-IV[6] is ubiquitously expressed, with the highest levels found in the kidney and the lower levels in liver, pancreas, placenta, thymus, spleen, epithelial cells, vascular endothelium, and lymphoid and myeloid cells. A soluble form is shed into the circulation. Substrate specificity studies point to DPP-IV's strong preference for cleavage of peptides containing a proline residue in P_1,[7] though interestingly GLP-1 and related glucagon family members contain alanine at this position. A wide range of substituents are allowed at P_2, and there is also little preference for specific residues on the prime side except that proline and hydroxyproline are disfavored at P_1'.

While a large number of peptides are cleaved by DPP-IV in vitro,[8] very few have been shown to be endogenous substrates based on the following stringent criteria: (i) cleavage occurs in vitro at the penultimate residue; (ii) cleavage products are observed in vivo but are absent in the presence of a selective inhibitor or in DPP-IV$^{-/-}$ mice; (iii) cleavage is the major route of clearance of the peptide. GLP-1 meets these criteria, as does the incretin hormone glucose-dependent insulinotropic polypeptide (GIP, also known as gastric inhibitory peptide).[9] GIP, which is secreted in the proximal gut in response to food, stimulates insulin secretion in a glucose-dependent manner and is believed to account for approximately half the incretin response in healthy humans.[9c] Unlike GLP-1, the insulinotropic effects of GIP are reduced in type 2 diabetics, and this may contribute to the reduced incretin effects in these patients.

DPP-IV inhibitors evoke decreases in glucose excursion following an oral glucose challenge. Recent studies demonstrate efficacy of inhibitors in mice lacking one or both of the receptors for GLP-1 and GIP.[10] Clearly there are other substrates in addition to these incretins that contribute to the biological activity of DPP-IV inhibitors. One potential candidate is pituitary adenylate cyclase-activating polypeptide (PACAP), a pancreatic neuropeptide that regulates lipid and carbohydrate metabolism. Intravenous administration of this peptide to mice results in rapid cleavage at the penultimate residue. The DPP-IV cleavage product is absent in DPP-IV$^{-/-}$ mice, suggesting a potential role for the enzyme in in vivo processing of PACAP.[11]

DPP-IV Inhibitor SAR

In light of DPP-IV's substrate specificity, it is not surprising that α-aminoacylpyrrolidine derivatives have been widely explored as DPP-IV inhibitors. The most potent of these contain an electrophile at the 2-position of the pyrrolidine ring (Figure 2), which forms an adduct with the active site serine. Irreversible inhibitors con-

† Phone: 732-594-5796. Fax: 732-594-5350. E-mail: ann_weber@merck.com.

10.1021/jm030628v CCC: $27.50
Published on Web 07/20/2004

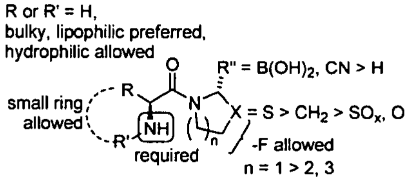


Figure 2. SAR of reversible α-aminoacylproline-derived DPP-IV inhibitors.

taining diphenylphosphonate esters[12] and *O*-acylhydroxamic acids[13] have been identified. A boronic acid moiety provides highly potent inhibitors that are slowly reversible,[14] but the most extensively studied agents are those containing a nitrile at this position. Replacement of the pyrrolidine with thiazolidine gives derivatives with increased potency; however, larger rings (e.g., piperidine, homopiperidine) or those containing other heteroatoms (e.g., oxazolidine) are less potent.[15] With the exception of fluorine, substituents on the pyrrolidine ring are not well-tolerated. In the thiazolidine series, oxidation of the sulfur to sulfoxide or sulfone leads to a decrease in activity.

A basic amine at P_2 is strictly required for inhibition. Consistent with the substrate specificity studies, a wide range of side chains at P_2 are tolerated, including bulky, lipophilic groups, and those containing polar functionality. Branching at this position is preferred, and of the simple amino acid substituents, isoleucyl, and cyclopentylglycyl, and cyclohexylglycyl provide the most potent inhibitors.[16]

Peptides containing sarcosine at P_2 are also substrates for DPP-IV, and this knowledge led to the exploration of N-substituted glycine derivatives as DPP-IV inhibitors.[17] Like their α-substituted amino acid counterparts, these inhibitors tolerate both straight-chain and cyclic substituents at this position, with polar and lipophilic side chains including the very bulky adamantyl group. Two derivatives from this class have been studied in the clinic: DPP728 (**1a**) and LAF237 (**2**, IC_{50} = 22 and 3.5 nM, respectively; Chart 1).

Substituents on nitrogen appear to fill the same S_2 site as those on the α-carbon. Indeed *N*,α-bis-substituted analogues show greatly decreased potency.[17c] Small rings bridging carbon and nitrogen are tolerated, including proline at P_2. A series of tetrahydroisoquinoline-3-carbonylcyanopyrrolidine derivatives are also reported to have good DPP-IV inhibitory activity (e.g., **3**; IC_{50} = 4 nM).[18]

Because of the presence of the required basic amine, electrophile-containing inhibitors generally exhibit a high degree of solution instability. This may contribute in part to the relatively short half-life of these derivatives in vivo. Product-like inhibitors, those lacking an electrophile, have also been developed. While more stable, they typically are much less potent than the corresponding nitriles. One of these, *threo*-isoleucylthiazolidide or P32/98 (**4**, K_i = 126 nM),[19] was advanced to clinical trials. Cyclohexylglycylpyrrolidide (**5**, K_i = 64 nM) is among the most potent DPP-IV inhibitors lacking an electrophilic serine trap.[16] Recently, derivatives with substitution at the 4-position of the cyclohexyl ring were reported to have increased potency. The 4-(2,2,2-trifluoroethyl)sulfonamidophenylsulfonylamino derivative **6** has an IC_{50} of 2.6 nM and is >1000-fold selective over the related prolyl peptidase QPP (quiescent cell proline dipeptidase).[20]

The amide bond is not strictly required for potency, and inhibitors such as **7** containing a fluoroolefin amide bond replacement have been reported.[21] A number of heterocyclic structures devoid of peptide-like character have also been shown to inhibit DPP-IV. These include xanthine derivatives such as **8** (IC_{50} = 5 nM)[22] and isoquinoline[23] and isoquinolone[24] derivatives **9** and **10** (IC_{50} = 320 and 250 nM, respectively).

The X-ray crystal structure of DPP-IV bound to an inhibitor has recently been solved by several laboratories.[25] The enzyme is a homodimer. Each subunit comprises an α/β-hydrolase domain and an eight-bladed β-propeller domain. A large cavity, roughly 30−45 Å wide, is located between the two domains, and inhibitors bind to a small pocket in this cavity, with key residues from both domains making up the binding site. The basic amine forms a salt bridge with Glu205 and, in some cases, Glu206 from the β-propeller domain. Arg125, also from that domain, stabilizes the amide carbonyl moiety. The proline binding pocket is formed by a group of hydrophobic, primarily aromatic residues from the α/β-hydrolase domain, leaving little room to accommodate larger substituents at that position. The active site serine, Ser630, forms an imidate with the nitrile as predicted. The S_2 site is bounded by Ser209, Phe357, and Arg358. This pocket readily accommodates the 5-iodopyrid-2-ylaminoethyl side chain of glycine derivative **1b** and the 4-iodobenzyl side chain of phenylalanine derivative **11**. Thus, the crystal structures provide ready explanations for the structural requirements of the basic amine and pyrrolidine residues, the increased potency of the nitrile-containing analogues, and the less stringent requirements for moieties at P_2. It remains to be seen how this information will be used to design the next generation of DPP-IV inhibitors.

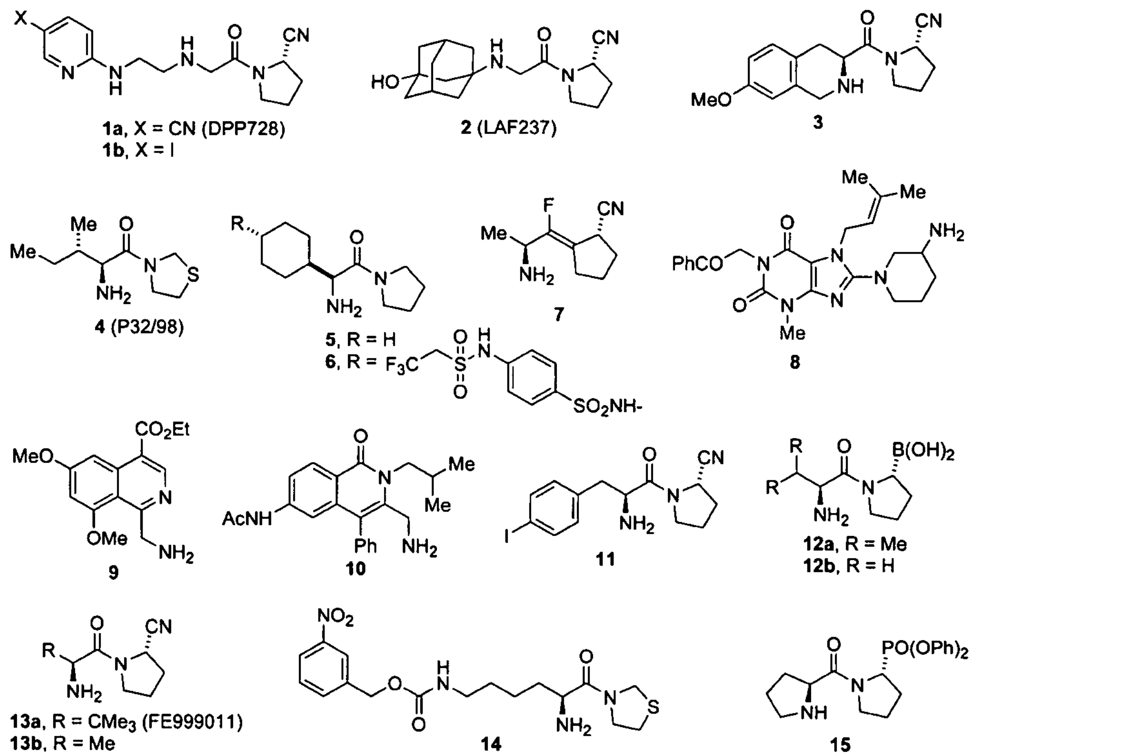
Related Enzymes

In the current inhibitor designs, selectivity over a wide range of proteases is generally possible. The stringent requirement for a basic amine provides inhibitors with selectivity over proline endopeptidases, while the preference for pyrrolidine at P_1 provides selectivity over other aminopeptidases.[17a] However, inhibitors that are thought to be specific for DPP-IV may in fact inhibit other enzymes in the "DPP-IV activity and/or structural homologue" (DASH) family.[26] Several family members have only recently been described, and thus, selectivity data are not generally available.

Fibroblast activation protein α (FAP-α, also known as seprase) shares the highest sequence homology to DPP-IV. This enzyme, which is found in tissue remodeling sites and tumors but not in healthy adult tissue, may be important in wound healing and cancer invasion. Recently the DPP-IV inhibitor Val-boro-Pro (**12a**) was shown to inhibit both DPP-IV and FAP.[27] This compound's ability to stimulate regeneration of neutrophils following cyclophosphamide treatment, originally attributed to DPP-IV inhibition, was noted in both wild-type and DPP-IV-deficient mice, suggesting that another prolyl peptidase such as FAP-α might be responsible for the observed biological activity.

Two other closely related DPP-IV-like proteins, DPP8[28] and DPP9,[29] are soluble, cytoplasmic enzymes that are

Chart 1. Structures of DPP-IV Inhibitors



ubiquitously expressed. DPP8 is up-regulated on activated T-cells, while high levels of DPP9 are found in skeletal muscle, heart, and liver. The latter enzyme was originally reported to lack peptidase activity, but that has recently been refuted.[29b] The biological function of both proteins is currently unknown, as is the degree to which "selective" DPP-IV compounds inhibit them.

Quiescent cell proline dipeptidase (QPP), renamed DPP7, appears to be identical to DPP II[30] and is located in intracellular vesicles. This enzyme shares sequence homology to prolyl carboxypeptidase but has DPP-IV-like, prolyl aminodipeptidase activity. Val-boro-Pro (**12a**), which inhibits DPP-IV with a K_i of 2 nM, is a 125 nM inhibitor of QPP.[31] Treatment of peripheral blood monocytes with this inhibitor induces apoptosis in quiescent but not activated lymphocytes. This effect is seen in T-cells lacking DPP-IV and has thus been attributed to the compound's ability to inhibit QPP in these cells. Selective inhibitors of QPP have been reported and may be useful in determining the biological role of this enzyme.[32]

Selectivity over these related enzymes may prove to be important for identifying safe and well tolerated inhibitors. In addition, caution must be used in interpreting studies with DPP-IV inhibitors because it is clear that in some cases, biological effects have been incorrectly attributed to DPP-IV inhibition.

Preclinical Proof of Concept Studies

There is a growing body of evidence to suggest that inhibition of DPP-IV will have therapeutic effects in treating diabetes. DPP-IV$^{-/-}$ mice show decreased blood glucose levels accompanied by an increase in insulin following an oral glucose challenge.[33] In addition, Fischer F344/DuCrj rats, which have a natural point mutation in DPP-IV affecting trafficking of the enzyme, have greatly reduced plasma DPP-IV activity and show improved glucose tolerance.[34]

Acute inhibition of DPP-IV by small-molecule inhibitors leads to increases in plasma GLP-1 levels and decreases in glucose excursion following an oral glucose challenge in both normal mice and rats and in animal models of diabetes and impaired glucose tolerance, including diet-induced obese (DIO) mice[35] and Zucker fatty rats.[36] An increase in insulin precedes the decrease in blood glucose, suggesting that the mechanism of glucose lowering is increased insulin secretion. The indirect effect of these compounds is also supported by the observation that DPP-IV inhibitors have no effect on glucose-stimulated insulin secretion in isolated islets.[35] In db/db mice, DPP-IV inhibition reduces glucose excursion in young animals but not in older animals with impaired β-cell function and pronounced insulin resistance.[37] Data from this acute study thus suggest that DPP-IV inhibitors may not prove to be efficacious in advanced diabetics but rather in patients with early stages of the disease.

A number of chronic animal studies provide support for the use of DPP-IV inhibitors in the long-term treatment of diabetes. Chronic administration of DPP-IV inhibitor isoleucylthiazolidide (**4**) to VDF Zucker rats, a model characterized by mild hyperglycemia, hyperinsulinemia, and insulin resistance, resulted in a decrease in the 24 h glucose profile and a progressive decrease in both fasting and peak blood glucose levels.[38] Following 12 weeks of treatment, an increase in glucose uptake in soleus muscle was evident as was an increase in the rate of insulin secretion in perfused pancreases from treated animals. The first-phase insulin response, which was absent in controls, was restored in the treated animals. Euglycemic–hyperinsulinemic clamp

studies showed an increase in glucose disposal and a decrease in hepatic glucose output.[39]

Chronic studies in Zucker diabetic fatty (ZDF) rats, which become overtly diabetic at about 8 weeks of age, suggest that DPP-IV inhibition may delay the development of disease.[40] Treatment of 6 week old animals with the potent DPP-IV inhibitor FE 999011 (**13a**, $IC_{50} = 7$ nM) delayed the onset of hyperglycemia from day 8 in vehicle-treated animals to day 15 in rats dosed with 10 mg/kg FE 999011 QD. In ZDF rats dosed with 10 mg/kg b.i.d., the onset was delayed to day 24, suggesting that near-complete, 24 h inhibition of DPP-IV is necessary to obtain maximal efficacy. Free fatty acids and triglycerides were maintained below levels considered toxic to β-cells in the b.i.d.-treated animals; thus, preservation of islet function is a possible mechanism for the delayed onset of diabetes.

There is additional evidence to suggest that chronic DPP-IV inhibition may preserve or restore islet function. In isolated islets from DIO mice treated with DPP728 (**1a**), an increase in insulin response at medium glucose concentrations was noted while maximal glucose-stimulated insulin secretion was not effected.[41] This was accompanied by an increase in GLUT-2, a β-cell glucose transporter. While there was no effect on body weight in the treated animals, islet size was normal; thus, DPP-IV inhibition appears to counteract the increase in islet size that is typically seen in animals fed a high-fat diet.

In a recent report,[42] Wistar rats were treated chronically with isoleucylthiazolidide (**4**) beginning 1 week before or 1 week after administration of streptazotocin (STZ), a toxin that destroys pancreatic β-cells. In the early treatment group, postprandial glucose levels were less than both the late treatment and control STZ-treated groups and plasma insulin levels were higher. The early treatment group showed an increase in glucose-stimulated insulin secretion in perfused pancreas studies and an increase in β-cell number, indicating a cytoprotective effect of DPP-IV inhibition. After 6 weeks, the late treatment group also showed a progressive decrease in glucose and an increase in insulin. Both early and late treatment groups had increases in the smallest size subset of islets relative to STZ-treated controls, with near-normal β-cell fractions, suggesting β-cell regeneration or islet neogenesis.

Taken together, data from preclinical studies indicate that treatment with a DPP-IV inhibitor may provide improved efficacy in the early stages of diabetes and may delay progression of the disease. The potential for preservation and regeneration of β-cells suggests a role for DPP-IV inhibition even in the late stages of diabetes.

Clinical Studies

Preliminary clinical results have been disclosed on three DPP-IV inhibitors: isoleucylthiazolidide (**4**), DPP728 (**1a**), and LAF237 (**2**). Isoleucylthiazolidide was reported to be safe and well-tolerated in normal volunteers at doses up to 240 mg.[43] DPP-IV was inhibited in a dose-dependent manner. In an open label study in diabetic patients a decrease in glucose excursion following an OGTT was seen at a dose of 60 mg.[44] Increases in both active GLP-1 and GIP were noted.

Patients with mild diabetes were treated for 4 weeks with DPP728.[45] Because of the short half-life of this compound in humans ($t_{1/2} = 50$ min), the total daily dose of 300 mg was divided (150 mg b.i.d. and 100 mg t.i.d.). Both dosing regimens led to decreases in fasting and prandial glucose and mean 24 h glucose relative to placebo. While the drug was generally well-tolerated, transient pruritus localized to the palms was noted in treated subjects, perhaps due to potentiation of an unknown bioactive peptide such as substance P.

Development of DPP728 has been discontinued in favor of LAF237, which has a profile suitable for once daily dosing. Following administration to diabetic patients at a dose of 100 mg qd for 4 weeks, decreases in fasting glucose, postprandial glucose, and postprandial glucagon levels were seen.[46]

While these initial clinical studies appear promising, the long-term safety, efficacy, and durability of DPP-IV inhibitor treatment remain to be established.

Additional Opportunities and Potential Pitfalls

With its mechanism of glucose-dependent insulin biosynthesis and secretion, DPP-IV inhibition provides the potential opportunity for excellent synergy with existing diabetes treatments. Combination therapy with insulin sensitizing agents such as PPARγ agonists and agents that control hepatic glucose output such as biguanides may prove to be particularly effective. The former combination has been explored in obese Zucker rats.[47] Following a 10 day treatment, a synergistic effect was observed with DPP-IV inhibitor LAF237 (**2**) and PPARγ agonist pioglitazone, in particular, on the increase in rate of glucose disposal.

Additional opportunities may exist in the treatment of diseases beyond diabetes. When fed a high-fat diet, DPP-IV$^{-/-}$ mice and DPP-IV-deficient Fischer F344/DuCrj rats are resistant to weight gain, suggesting a role for DPP-IV inhibition in the treatment of obesity.[48] Food intake in both the DPP-IV$^{-/-}$ mice and Fischer rats is reduced. Pair-fed wild-type mice weigh more than the DPP-IV$^{-/-}$ animals; thus, a metabolic component may exist. In animal studies with DPP-IV inhibitors, however, weight loss is typically minimal or not seen at all. While it is unclear how these results will translate into clinical findings, DPP-IV inhibitors are not likely to cause the weight gain that is often associated with current diabetes medications.

Potentiation of endogenous substrates beyond GLP-1 and GIP may provide further therapeutic opportunities. For example, GLP-2, an intestinal growth factor released in the gut in response to nutrient ingestion, is inactivated by DPP-IV in vivo in rats.[49] Thus, inhibition of DPP-IV may prove to be useful in the treatment of intestinal injury and disease. This indication remains to be fully explored. DPP-IV is also thought to regulate endomorphin-2, a tetrapeptide (Tyr-Pro-Phe-Phe-NH_2) with high affinity for the μ opioid receptor.[50] The icv administration of DPP-IV inhibitors alanylpyrrolidine-2-nitrile[50a] (**13b**) and DiprotinA[50b] (Ile-Pro-Ile) evoked dose-dependent potentiation of endomorphin-2 induced analgesia in the mouse paw withdrawal and tail flick models, respectively.

There are a variety of other substrates that DPP-IV cleaves in vitro.[8] These include chemokines such as RANTES, eotaxin, IP-10, and SDF-1α, neuropeptides such as substance P, β-casomorphin, NPY, and PYY,

and growth factors such as GRH. In many cases, the DPP-IV cleavage product is inactive or has altered receptor specificity. Additional studies are needed to determine whether these are in vivo substrates and, if so, whether DPP-IV inhibition will lead to desired therapeutic benefits in other diseases or will result in potentially toxic side effects.

DPP-IV has a number of proposed functions in addition to its role in metabolic control.[6] It binds adenosine deaminase, an enzyme important in the normal development and function of the immune system, and likely modulates local concentrations of adenosine. It also has a binding site for the extracellular matrix proteins collagen and fibronection, though its role as an adhesion molecule remains unclear. DPP-IV is identical to the cell surface marker CD26 and serves as a costimulatory molecule in T-cell activation. While DPP-IV inhibitors have been shown to inhibit T-cell activation in vitro, concentrations required for this activity are well above their reported K_i values; thus, a role for enzymatic activity in this function appears unlikely.

Because of the potential role of DPP-IV in the immune system, inhibitors have been studied in a number of immunological models. DPP-IV inhibitors Ala-boro-Pro (**12b**) and Lys(Z-NO_2) thiazolidide (**14**) as well as two natural product DPP-IV inhibitors have been shown to inhibit hind paw swelling in collagen- and alkyldiamine-induced models of arthritis in rats.[51] The irreversible inhibitor Pro-Pro-diphenyl phosphonate (**15**) prolonged allograph survival in a rat cardiac transplant model.[52] With the discovery of additional enzymes possessing DPP-IV-like activity, these results remain to be confirmed with more selective DPP-IV inhibitors.

Conclusions

Inhibition of DPP-IV is an attractive new approach to the treatment of type 2 diabetes. Because DPP-IV inhibitors stimulate insulin secretion in a glucose-dependent fashion, the potential for hypoglycemic side effects is minimal. The lack of weight gain, and potential for weight loss, with DPP-IV inhibitor treatment provides another potential benefit to diabetics, the vast majority of whom are obese. Finally, recent data suggesting restorative effects on pancreatic islets provide hope that DPP-IV inhibitors will slow or perhaps reverse the course of disease. The promise of this treatment remains to be realized as potent and selective inhibitors progress through clinical studies.

Biography

Ann E. Weber received her B.S. degree in Chemistry from the University of Notre Dame and Ph.D. degree in Organic Synthesis from Harvard University, working in the laboratories of Professor David Evans. She joined Merck & Co. in 1987. Currently she is a Senior Director in the Department of Medicinal Chemistry, focusing primarily on the discovery of novel chemotherapeutics for the treatment of metabolic disorders.

References

(1) Creutzfeldt, W. The Incretin Concept Today. *Diabetologia* **1979**, *16*, 75–85.

(2) Knudsen, L. B. Glucagon-like Peptide-1: The Basis of New Class of Treatment for Type 2 Diabetes. *J. Med. Chem.* **2004**, *47*, 4128–4134.

(3) (a) Mentlein, R.; Ballwitz, B.; Schmidt, W. E. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7–36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur. J. Biochem.* **1993**, *214*, 829–835. (b) Kieffer, T. J.; McIntosh, C. H. S.; Pederson, T. A. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrine* **1995**, *136*, 3585–3596. (c) Deacon, C. F.; Nauck, M. A.; Toft-Nielsen, M.; Pridal, L.; Willms, B.; Holst, J. J. Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH_2-terminus in type II diabetic patients and in healthy subjects. *Diabetes* **1995**, *44*, 1126–1131.

(4) (a) He, X. S.; Zhang, X.; Zhao, H.; DeSimone, R.; Thurkauf, A. 1-Benzylimidazole derivatives. U.S. 6,482,844, 2002. (b) Desimone, R. W.; Hutchison, A. Aryl and heteroaryl fused aminoalkyl-imidazole derivatives and their use as antidiabetics. WO 00/59887, October 12, 2000. (c) Teng, M.; Truesdale, L.; Bhumralkar, D.; Kiel, D.; Johnson, M. D.; Thomas, C.; Jørgensen, A.; Madsen, P.; Olesen, P. Non-peptide GLP-1 Agonists. WO 00/42026, July 20, 2000.

(5) (a) Holst, J. J.; Deacon, C. F. Inhibition of the activity of dipeptidyl-peptidase IV as a treatment for type 2 diabetes. *Diabetes* **1998**, *47*, 1663–1670. (b) Augustyns, K.; Bal, G.; Thonus, G.; Belyaev, A.; Zhang, X. M.; Bollaert, W.; Lambeir, A. M.; Durinx, C.; Goossens, F.; Haemers, A. The unique properties of dipeptidyl-peptidase IV (DPP IV/CD26) and the therapeutic potential of DPP IV inhibitors. *Curr. Med. Chem.* **1999**, *6*, 311–327. (c) Villhauer, E. B.; Coppola, G. M.; Hughes, T. E. DPP-IV inhibition and therapeutic potential. *Annu. Rep. Med. Chem.* **2001**, *36*, 191–200. (d) Drucker, D. J. Therapeutic potential of dipeptidyl peptidase IV inhibitors for the treatment of type 2 diabetes. *Expert Opin. Invest. Drugs* **2003**, *12*, 87–100. (e) Wiedeman, P. E.; Trevillyan, J. M. Dipeptidyl peptidase IV inhibitors for the treatment of impaired glucose tolerance and type 2 diabetes. *Curr. Opin. Invest. Drugs* **2003**, *4*, 412–420. (f) Augustyns, K.; Van der Veken, P.; Senten, K.; Haemers, A. Dipeptidyl peptidase IV inhibitors as new therapeutic agents for the treatment of type 2 diabetes. *Expert Opin. Ther. Pat.* **2003**, *13*, 499–510.

(6) (a) De Meester, I.; Korom, S.; Van Damme, J.; Scharpé, S. CD26, let it cut or cut it down. *Immunol. Today* **1999**, *20*, 367–375. (b) Hildebrandt, M.; Reutter, W.; Arck, P.; Rose, M.; Klapp, B. F. A guardian angel: the involvement of dipeptidyl peptidase IV in psychoneuroendocrine function, nutrition and immune defense. *Clin. Sci.* **2000**, *99*, 93–104. (c) Boonacker, E.; Van Noorden, C. J. F. The multifunctional or moonlighting protein CD26/DPPIV. *Eur. J. Cell Biol.* **2003**, *82*, 53–73.

(7) (a) Püschel, G.; Mentlein, R.; Heymann, E. Isolation and characterization of dipeptidyl peptidase IV from human placenta. *Eur. J. Biochem.* **1982**, *126*, 359–365. (b) Heins, J.; Welker, P.; Schönlein, C.; Born, I.; Hartrodt, B.; Neubert, K.; Tsuru, D.; Barth, A. Mechanism of proline-specific proteinases: (I) substrate specificity of dipeptidyl peptidase IV from pig kidney and proline-specific endopeptidase from *Flavobacterium meningosepticum*. *Biochim. Biophys. Acta* **1988**, *954*, 161–169. (c) Rahfeld, J.; Schutkowski, M.; Faust, J.; Neubert, K.; Barth, A.; Heins, J. Extended investigation of the substrate specificity of dipeptidyl peptidase IV from pig kidney. *Biol. Chem. Hoppe–Seyler* **1991**, *372*, 313–318.

(8) Mentlein, R. Dipeptidyl-peptidase IV (CD26)—role in the inactivation of regulatory peptides. *Regul. Pept.* **1999**, *85*, 9–24.

(9) (a) Deacon, C. F.; Nauck, M. A.; Meier, J.; Hücking, K.; Holst, J. J. Degradation of endogenous and exogenous gastric inhibitory polypeptide in healthy and in type 2 diabetic subjects as revealed using a new assay for the intact peptide. *J. Clin. Endocrinol. Metab.* **2000**, *85*, 3575–3581. (b) Meier, J. J.; Nauck, M. A.; Schmidt, W. E.; Gallwitz, B. Gastric inhibitory polypeptide: the neglected incretin revisited. *Regul. Pept.* **2002**, *107*, 1–13. (c) Vilsbøll, T.; Krarup, T.; Madsbad, S.; Holst, J. J. Both GLP-1 and GIP are insulinotropic at basal and postprandial glucose levels and contribute nearly equally to the incretin effect of a meal in healthy subjects. *Regul. Pept.* **2003**, *114*, 115–121.

(10) Hansotia, T.; Baggio, L.; Yamada, Y.; Tsukiyama, K.; Preitner, F.; Thorens, B.; Seino, Y.; Drucker, D. Combined genetic disruption of dual incretin receptor signaling pathways reveals novel glucose lowering properties of DPP-IV inhibitors in mice. *Diabetes* **2003**, *52* (S1), A77.

(11) Zhu, L.; Tamvakopoulos, C.; Xie, D.; Dragovic, J.; Shen, X.; Fenyk-Melody, J. E.; Schmidt, K.; Bagchi, A.; Griffin, P. R.; Thornberry, N. A.; Sinha Roy, R. The role of dipeptidyl peptidase IV in the cleavage of glucagon family peptides. *J. Biol. Chem.* **2003**, *278*, 22418–22423.

(12) Senten, K.; Daniëls, L.; Van der Veken, P.; De Meester, I.; Lambeir, A. M.; Scharpé, S.; Haemers, A.; Augustyns, K. Rapid parallel synthesis of dipeptide diphenyl phosphonate esters as inhibitors of dipeptidyl peptidases. *J. Comb. Chem.* **2003**, *5*, 336–344.

(13) Demuth, H. U.; Baumgrass, R.; Schaper, C.; Fischer, G.; Barth, A. Dipeptidyl peptidase IV—inactivation with *N*-peptidyl-*O*-aryl hydroxylamines. *J. Enzyme Inhib.* **1988**, *2*, 129–142.
(14) Snow, R. J.; Bachovchin, W. W. Boronic acid inhibitors of dipeptidyl peptidase IV: a new class of immunosuppressive agents. *Adv. Med. Chem.* **1995**, *3*, 149–177.
(15) (a) Ashworth, D. M.; Atrash, B.; Baker, G. R.; Baxter, A. J.; Jenkins, P. D.; Jones, D. M.; Szelke, M. 4-Cyanothiazolidides as very potent, stable inhibitors of dipeptidyl peptidase IV. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2745–2748. (b) Augustyns, K. J. L.; Lambeir, A. M.; Borloo, M.; De Meester, I.; Vedernikova, I.; Vanhoof, G.; Hendriks, D.; Scharpé, S.; Haemers, A. Pyrrolidides: synthesis and structure–activity relationship as inhibitors of dipeptidyl peptidase IV. *Eur. J. Med. Chem.* **1997**, *32*, 301–309.
(16) Ashworth, D. M.; Atrash, B.; Baker, G. R.; Baxter, A. J.; Jenkins, P. D.; Jones, D. M.; Szelke, M. 2-Cyanopyrrolidides as potent, stable inhibitors of dipeptidyl peptidase IV. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1163–1166.
(17) (a) Hughes, T. E.; Mone, M. D.; Russell, M. E.; Weldon, S. C.; Villhauer, E. B. (1-[[[2-[(5-Cyanopyridin-2-yl)amino]ethyl]amino]-acetyl]-2-cyano-(*S*)-pyrrolidine), a slow-binding inhibitor of dipeptidylp peptidase IV. *Biochemistry* **1999**, *38*, 11597–11603. (b) Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Dunning, B. E.; Mangold, B. L.; Mone, M. D.; Russell, M. E.; Weldon, S. C.; Hughes, T. E. 1-[2-[(5-Cyanopyridin-2-yl)amino]ethylamino]-acetyl-2-(*S*)-pyrrolidinecarbonitrile: a potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties. *J. Med. Chem.* **2002**, *45*, 2362–2365. (c) Villhauer, E. B.; Brinkman, J. A.; Naderi, G.; Burkey, B. F.; Dunning, B. E.; Prasad, K.; Mangold, B. L.; Russell, M. E.; Hughes, T. E. 1-[[(3-Hydroxy-1-adamantyl)amino]acetyl]-2-cyano-(S)-pyrrolidine: A potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties. *J. Med. Chem.* **2003**, *46*, 2774–2789.
(18) Damon, R. Tetrahydroisoquinoline 3-carboxamide derivatives. U.S. 6,172,081, 2001.
(19) (a) Schön, E.; Born, I.; Demuth, H. U.; Faust, J.; Neubert, K.; Steinmetzer, T.; Barth, A.; Ansorge, S. Dipeptidyl peptidase IV in the immune system. *Biol. Chem. Hoppe–Seyler* **1991**, *372*, 305–311. (b) Stöckel-Maschek, A.; Mrestani-Klaus, C.; Stiebitz, B.; Demuth, H. U.; Neubert, K. Thioxo amino acid pyrrolidides and thiazolidides: new inhibitors of proline specific peptidases. *Biochim. Biophys. Acta* **2000**, *1479*, 15–31.
(20) Parmee, E. R.; He, J.; Mastracchio, A.; Edmondson, S. D.; Colwell, L.; Eiermann, G.; Feeney, W. P.; Habulihaz, B.; He, H.; Kilburn, R.; Leiting, B.; Lyons, K.; Marsilio, F.; Patel, R.; Petrov, A.; Di Salvo, J.; Wu, J. K.; Thornberry, N. A.; Weber, A. E. 4-Amino cyclohexylglycine analogs as potent dipeptidyl peptidase IV inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 43–46.
(21) Zhao, K.; Lim, D. S.; Funaki, T.; Welch, J. T. Inhibition of dipeptidyl peptidase IV (DPP IV) by 2-(2-amino-1-fluoro-propylidine)-cyclopentanecarbonitrile, a fluoroolefin containing peptidomimetic. *Bioorg. Med. Chem. Lett.* **2003**, *11*, 207–215.
(22) (a) Himmelsbach, F.; Mark, M.; Eckhardt, M.; Langkopf, E.; Mater, R.; Lotz, R. Xanthine derivatives, production and use thereof as a medicament. WO02/068420, 2002. (b) Branner, K. A.; Carr, R. D.; Christiansen, L. B.; Elm, T.; Ribel, U. A structurally novel dipeptidyl peptidase IV inhibitor: 7-benzyl-1,3-dimethyl-8-piperazinoxanthine. Presented at the 28th National Medicinal Chemistry Symposium, June 8–12, 2002; Abstract 72.
(23) Coppola, G. M.; Zhang, Y. L.; Schuster, H. F.; Russell, M. E.; Hughes, T. E. 1-Aminomethylisoquinoline-4-carboxylates as novel dipeptidyl peptidase IV inhibitors. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1555–1558.
(24) Oi, S.; Ikedou, K.; Takeuchi, K.; Ogino, M.; Banno, Y.; Tawada, H.; Yamane, T. Fused heterocyclic compounds. WO02/062764, 2002.
(25) (a) Rasmussen, H. B.; Branner, S.; Wiberg, F. C.; Wagtmann, N. Crystal structure of human dipeptidyl peptidase IV/CD26 in complex with a substrate analog. *Nat. Struct. Biol.* **2003**, *10*, 19–25. (b) Engel, M.; Hoffmann, T.; Wagner, L.; Wermann, M.; Heiser, U.; Kiefersauer, R.; Huber, R.; Bode, W.; Demuth, H.-U.; Brandstetter, H. The crystal structure of dipeptidyl peptidase IV (CD26) reveals its functional regulation and enzymatic mechanism. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 5063–5068. (c) Oefner, C.; D'Arcy, A.; Mac Sweeney, A.; Pierau, S.; Gardiner, R.; Dale, G. E. High-resolution structure of human apo dipeptidyl peptidase IV/CD26 and its complex with 1-[({2-[(5-iodopyridin-2-yl)amino]ethyl}amino)acetyl]-2-cyano-(*S*)-pyrrolidine. *Acta Crystallogr.* **2003**, *D59*, 1206–1212. (d) Thoma, R.; Löffler, B.; Stihle, M.; Huber, W.; Ruf, A.; Hennig, M. Structural basis of proline-specific exopeptidase activity as observed in human dipeptidyl peptidase-IV. *Structure* **2003**, *11*, 947–959.
(26) (a) Rosenblum, J. S.; Kozarich, J. W. Prolyl peptidases: a serine protease subfamily with high potential for drug discovery. *Curr. Opin. Chem. Biol.* **2003**, *7*, 1–9. (b) Šedo, A.; Malik, R. Dipeptidyl peptidase IV-like molecules: homologous proteins or homologous activities? *Biochim. Biophys. Acta* **2001**, *1550*, 107–116. (c) Cunningham, D. F.; O'Connor, B. Proline specific peptidases. *Biochim. Biophys. Acta* **1997**, *1343*, 160–186.
(27) Jones, B.; Adams, S.; Miller, G. T.; Jesson, M. I.; Watanabe, T.; Wallner, B. P. Hematopoietic stimulation by a peptidyl peptidase inhibitor reveals a novel regulatory mechanism and therapeutic treatment for blood cell deficiencies. *Blood* **2003**, *102*, 1641–1648.
(28) Abbott, C. A.; Yu, D. M. T.; Woollatt, E.; Sutherland, G. R.; McCaughan, G. W.; Gorrell, M. D. Cloning, expression and chromosomal localization of a novel human dipeptidyl peptidase (DPP) IV homolog, DPP8. *Eur. J. Biochem.* **2000**, *267*, 6140–6150.
(29) (a) Olsen, C.; Wagtmann, N. Identification and characterization of human DPP9, a novel homologue of dipeptidyl peptidase IV. *Gene* **2002**, *299*, 185–193. (b) Qi, S. Y.; Riviere, P. J.; Trojnar, J.; Junien, J. L.; Akinsanya, K. O. Cloning and characterization of dipeptidyl peptidase 10, a new member of an emerging subgroup of serine proteases. *Biochem. J.* **2003**, *373*, 179–189.
(30) Leiting, B.; Pryor, K. D.; Wu, J. K.; Marsilio, F.; Patel, R. A.; Craik, C. S.; Ellman, J. A.; Cummings, R. T. Thornberry, N. A. Catalytic properties and inhibition of proline-specific dipeptidyl peptidases II, IV and VII. *Biochem. J.* **2003**, *371*, 525–532.
(31) Underwood, R.; Chiravuri, M.; Lee, H.; Schmitz, T.; Kabcenell, A. K.; Yardley, K.; Huber, B. T. Sequence, purification, and cloning of an intracellular serine protease, quiescent cell proline dipeptidase. *J. Biol. Chem.* **1999**, *274*, 34053–34058.
(32) Senten, K.; Van der Veken, P.; Bal, G.; De Meester, I.; Lambeir, A. M.; Scharpé, S.; Bauvois, B.; Haemers, A.; Augustyns, K. Development of Potent and Selective Dipeptidyl Peptidase II Inhibitors. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2825–2828.
(33) Marguet, D.; Baggio, L.; Kobayashi, T.; Bernard, A.-M.; Pierres, M.; Nielsen, P. F.; Ribel, U.; Watanabe, T.; Drucker, D. J.; Wagtmann, N. Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6874–6879.
(34) Nagakura, T.; Yasuda, N.; Yamazaki, K.; Ikuta, H.; Yoshikawa, S.; Asano, O.; Tanaka, I. Improved glucose tolerance via enhanced glucose-dependent insulin secretion in dipeptidyl peptidase IV-deficient Fischer rats. *Biochem. Biophys. Res. Commun.* **2001**, *284*, 501–506.
(35) Ahrén, B.; Holst, J. J.; Mårtensson, H.; Balkan, B. Improved glucose tolerance and insulin secretion by inhibition of dipeptidyl peptidase IV in mice. *Eur. J. Pharmacol.* **2000**, *404*, 239–245.
(36) (a) Pederson, R. A.; White, H. A.; Schlenzig, D.; Pauly, R. P.; McIntosh, C. H.; Demuth, H.-U. Improved glucose tolerance in Zucker fatty rats by oral administration of the dipeptidyl peptidase IV inhibitor isoleucine thiazolidide. *Diabetes* **1998**, *47*, 1253–1258. (b) Balkan, B.; Kwasnik, L.; Miserendino, R.; Holst, J. J.; Li, X. Inhibition of dipeptidyl peptidase IV with NVP-DPP728 increases plasma GLP-1 (7–36 amide) concentrations and improves oral glucose tolerance in obese Zucker rats. *Diabetologia* **1999**, *42*, 1324–1331.
(37) Nagakura, T.; Yasuda, N.; Yamazaki, K.; Ikuta, H.; Tanaka, I. Enteroinsular axis of *db/db* mice and efficacy of dipeptidyl peptidase IV inhibition. *Metabolism* **2003**, *52*, 81–86.
(38) Pospisilik, J. A.; Stafford, S. G.; Demuth, H.-U.; McIntosh, C. H. S.; Pederson, R. A. Long-term treatment with dipeptidyl peptidase IV inhibitor improves hepatic and peripheral insulin sensitivity in the VDF Zucker rat. *Diabetes* **2002**, *51*, 2677–2683.
(39) Pospisilik, J. A.; Stafford, S. G.; Demuth, H.-U.; Brownsey, R.; Parkhouse, W.; Finegood, D. T.; McIntosh, C. H. S.; Pederson, R. A. Long-term treatment with dipeptidyl peptidase IV inhibitor P32/98 causes sustained improvements in glucose tolerance, insulin sensitivity, hyperinsulinemia, and β-cell responsiveness in VDF (*fa/fa*) Zucker rat. *Diabetes* **2002**, *51*, 943–950.
(40) Sudre, B.; Broqua, P.; White, R. B.; Ashworth, D.; Evans, D. M.; Haigh, R.; Junien, J.-L.; Aubert, M. L. Chronic inhibition of circulating dipeptidyl peptidase IV by FE 999011 delays the occurrence of diabetes in male Zucker diabetic fatty rats. *Diabetes* **2002**, *51*, 1461–1469
(41) Reimer, M. K.; Holst, J. J.; Ahrén, B. Long-term inhibition of dipeptidyl peptidase IV improves glucose tolerance and preserves islet function in mice. *Eur. J. Endocrinol.* **2002**, *146*, 717–727.
(42) Pospisilik, J. A.; Martin, J.; Doty, T.; Ehses, J. A.; Pamir, N.; Lynn, F. C.; Piteau, S.; Demuth, H.-U.; McIntosh, C. H. S.; Pederson, R. A. Dipeptidyl peptidase IV inhibitor treatment stimulates β-cell survival and islet neogenesis in streptozotocin-induced diabetic rats. *Diabetes* **2003**, *52*, 741–750.
(43) (a) Sorbera, L. A.; Revel, L.; Castañer, J. P32/98: Antidiabetic dipeptidyl-peptidase IV inhibitor. *Drugs Future* **2001**, *26*, 859–864. (b) Glund, K.; Hoffmann, T.; Demuth, H.-U.; Banke-Bochita, J.; Rost, K.-L.; Fuder, H. Single dose-escalation study to investigate the safety and tolerability of the DP IV inhibitor P32/98 in healthy volunteers. *Exp. Clin. Endocrinol. Diabetes* **2000**, *108*, 159.

(44) (a) Demuth, H.-U.; Hoffmann, T.; Glund, K.; McIntosh, C. H. S.; Pederson, R. A.; Fuecker, K.; Fischer, S.; Hanefeld, M. Single Dose Treatment of Diabetic Patients by the DP IV Inhibitor P32/98. *Diabetes* **2000**, *49* (S1), A102. (b) Hoffmann, T.; Wolf, R.; Rosche, F.; Fischer, S.; Hanefeld, M.; Pederson, R. A.; McIntosh, D. H. S.; Glund, K.; Demuth, H.-U. Kinetics of active and inactive plasma GIP in type 2 diabetic patients during treatment with the DP IV-inhibitor P32/98: direct determination by immunoprecipitation LC–MS. *Diabetes* **2002**, *51* (S2), A340.

(45) Ahrén, B.; Simonsson, E.; Larsson, H.; Landin-Olsson, M.; Torgeirsson, H.; Jansson, P.-A.; Sandqvist, M.; Båvenholm, P.; Efendic, S.; Eriksson, J. W.; Dickinson, S.; Holmes, D. Inhibition of dipeptidyl peptidase IV improves metabolic control over a 4-week study period in type 2 diabetes. *Diabetes Care* **2002**, *25*, 869–875.

(46) Ahrén, B.; Landin-Olsson, M.; Jansson, P.-A.; Eriksson, J.; Pacini, G.; Thomaseth, K.; Schweizer, A. The DPPIV inhibitor, LAF237, reduces fasting and postprandial glucose in subjects with type 2 diabetes over a 4 week period by increasing active GLP-1, sustaining insulin and reducing glucagon. *Diabetes* **2003**, *52* (S1), A15.

(47) Burkey, B. F.; Li, X.; Bolognese, L.; Russell, M.; Want, P. R.; Villhauer, E. B.; Hughes, T. E. Combination treatment of a DPP-IV inhibitor NVP-LAF237 with pioglitazone completely normalized glucose tolerance in adult obese Zucker rats. *Diabetes* **2002**, *51* (S2), A338–A339.

(48) (a) Conarello, S. L.; Li, Z.; Ronan, J.; Sinha Roy, R.; Zhu, L.; Jiang, G.; Liu, F.; Woods, J.; Zycband, E.; Moller, D. E.; Thornberry, N. A.; Zhang, B. B. Mice lacking dipeptidyl peptidase IV are protected against obesity and insulin resistance. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 6825–6830. (b) Yasuda, N.; Nagakura, T.; Yamazaki, K.; Inoue, T.; Tanaka, I. Improvement of high fat-diet-induced insulin resistance in dipeptidyl peptidase IV-deficient Fischer rats. *Life Sci.* **2002**, *71*, 227–238.

(49) Lovshin, J.; Drucker, D. J. New frontiers in the biology of GLP-2. *Regul. Pept.* **2000**, *90*, 27–32.

(50) (a) Shane, R.; Wilk, S.; Bodnar, R. J. Modulation of endomorphin-2-induced analgesia by dipeptidyl peptidase IV. *Brain Res.* **1999**, *815*, 278–286. (b) Sakurada, C.; Sakurada, S.; Hayashi, T.; Katsuyama, S.; Tan-No, K.; Sakurada, T. Degradation of endomorphin-2 at the supraspinal level in mice is initiated by dipeptidyl peptidase IV: an in vitro and in vivo study. *Biochem. Pharmacol.* **2003**, *66*, 653–661.

(51) (a) Tanaka, S.; Murakami, T.; Horikawa, H.; Sugiura, M.; Kawashima, K.; Sugita, T. Suppression of arthritis by the inhibitors of dipeptidyl peptidase IV. *Int. J. Immunopharmacol.* **1997**, *19*, 15–24. (b) Ohnuki, T.; Yamada, M.; Sugita, T. Novel dipeptidyl peptidase IV inhibitors with antiarthritic effects. *Drugs Future* **1999**, *24*, 665–670.

(52) (a) De Meester, I.; Belyaev, A.; Lambeir, A. M.; De Meyer, G. R. Y.; Van Osselaer, N.; Haemers, A.; Scharpé, S. In vivo inhibition of dipeptidyl peptidase IV activity by Pro-Pro-diphenyl-phosphonate (Prodipine). *Biochem. Pharmacol.* **1997**, *54*, 173–179. (b) Korom, S.; De Meester, I.; Stadlbauer, T. H. W.; Chandraker, A.; Schaub, M.; Sayegh, M. H.; Belyaev, A.; Haemers, A.; Scharpé, S.; Kupiec-Weglinski, J. W. Inhibition of CD26/dipeptidyl peptidase IV activity in vivo prolongs cardiac allograft survival in rat recipients. *Transplantation* **1997**, *63*, 1495–1500.

JM030628V

Full text provided by www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER

Glucagon-like peptide 1 and inhibitors of dipeptidyl peptidase IV in the treatment of type 2 diabetes mellitus

Jens Juul Holst* and Carolyn F Deacon

Proof-of-concept for the efficacy of a glucagon-like peptide 1 (GLP-1)-based therapy of patients with type 2 diabetes was provided in 2002 by means of prolonged continuous subcutaneous infusion of native GLP-1. Since then, several long-acting analogues of GLP-1, as well as inhibitors of dipeptidyl peptidase IV, the enzyme that rapidly inactivates endogenous GLP-1, have demonstrated efficacy in long term clinical trials.

Addresses
Department of Medical Physiology, University of Copenhagen, The Panum Institute, DK-2200 Copenhagen N, Denmark
*e-mail: holst@mfi.ku.dk

Current Opinion in Pharmacology 2004, 4:589–596

This review comes from a themed issue on Endocrine and metabolic diseases
Edited by Julia Buckingham and Brian Furman

Available online 14th October 2004

1471-4892/$ – see front matter

DOI 10.1016/j.coph.2004.08.005

Abbreviations

ADA	American Diabetes Association
DPP-IV	dipeptidyl peptidase IV
GLP-1	glucagon-like peptide 1
HbA1c	glycated haemoglobin
SU	sulfonylurea
T2DM	type 2 diabetes mellitus

Introduction

Glucagon-like peptide 1 (GLP-1) is a 30 amino acid peptide secreted by intestinal L-cells in response to meal ingestion. It functions as one of the incretin hormones; that is, the gut hormones that enhance nutrient-stimulated insulin secretion more than the nutrients themselves, if given intravenously [1]. In patients with type 2 diabetes mellitus (T2DM), the incretin effect is severely impaired or absent [2], and it is probable that this deficiency contributes to the deficient insulin secretion that characterizes T2DM [3]. The causes of the deficient incretin effect in patients with T2DM have been analysed [3] and seem to comprise an impaired secretion of GLP-1, an impaired sensitivity of the β-cell to the actions of GLP-1 (whereas the efficacy is at least partially preserved) [4], and an abolished effect of glucose-dependent insulinotropic polypeptide on second-phase insulin secretion [5]. In agreement with this, intravenous infusions of GLP-1 in near physiological amounts have been shown to almost completely normalize glucose metabolism in patients with T2DM [6,7]. Because of this, there is currently great interest in trying to develop GLP-1 as a new therapeutic agent for T2DM [8]. However, GLP-1 has many more actions than merely stimulating insulin secretion, and all of these seem to be expedient in the context of diabetes therapy.

Actions of GLP-1

GLP-1 potently stimulates insulin secretion in a strictly glucose-dependent manner. Binding of GLP-1 to the GLP-1 receptor of β-cells causes activation — via a stimulatory G protein — of adenylate cyclase, resulting in the formation of cAMP. Subsequent activation of protein kinase A and the cAMP-regulated guanine nucleotide exchange factor II (also known as Epac2) leads to a plethora of events including altered ion channel activity, intracellular calcium handling and enhanced exocytosis of insulin-containing granules [1]. The clinical implication of the dependence on blood glucose concentrations at or above normal fasting glucose levels is that GLP-1 is incapable of causing profound hypoglycaemia (except perhaps in the presence of sulfonylurea (SU) drugs; see below).

GLP-1 stimulates all steps of insulin biosynthesis, as well as insulin gene transcription [9], thereby providing continued and augmented supplies of insulin for secretion. Activation of PDX-1, a key regulator of islet growth and insulin gene transcription, might be involved [10]. In addition, GLP-1 upregulates genes for the cellular machinery involved in insulin secretion, such as glucokinase and glucose transporter-2 genes [10].

GLP-1 has been shown to have trophic effects on β-cells [11]: not only does it stimulate β-cell proliferation [12,13] but it also enhances the differentiation of new β-cells from progenitor cells in the pancreatic duct epithelium [14]. Proliferation was also induced in aging glucose-intolerant rats, with a resulting improvement in glucose tolerance [15]. Most recently, GLP-1 has been shown to inhibit apoptosis of β-cells, including human β-cells [16••]. Because the normal number of β-cells is maintained in a balance between apoptosis and proliferation, this observation is of considerable interest, and also raises the possibility that GLP-1 could be useful in conditions with increased β-cell apoptosis (e.g. when cells are exposed to the toxic effects of hyperglycaemia and hyperlipidaemia).

GLP-1 also strongly inhibits glucagon secretion. In patients with T2DM, there is fasting hyperglucagonemia as well as exaggerated glucagon responses to meal ingestion [17]; therefore, it is likely that the hyperglucagonemia contributes to the hyperglycemia of the patients. This effect could be as important as the insulinotropic effects.

Further important effects of GLP-1 include inhibition of gastrointestinal secretion and motility, notably gastric emptying [18,19]. This effect is desirable in patients with diabetes because the slower gastric emptying rate reduces postprandial glucose excursions; the clinical importance of this is evident from the use of another potent gastric inhibitor, amylin, for diabetes treatment [20].

GLP-1 also inhibits appetite and food intake. This has been demonstrated in both normal subjects, obese subjects and subjects with T2DM [21], and it is likely that GLP-1 is one of the physiological regulators of appetite and food intake.

GLP-1 has cardiovascular actions, as it has been known for some time that there are GLP-1 receptors in the heart [22]. A physiological function for these receptors was indicated in recent studies in mice lacking the GLP-1 receptor, which exhibit impaired left ventricular contractility and diastolic functions, as well as impaired responses to exogenous epinephrine [23•]. Recent studies in rats showed that GLP-1 protects the ischaemic and reperfused myocardium in rats by mechanisms independent of insulin [24••]. These findings could have important clinical implications. Thus, Nikolaidis *et al.* [25] studied patients treated with angioplasty after acute myocardial infarction, with postoperative left ventricular ejection fractions as low as 29%. In these patients, GLP-1 administration significantly improved the ejection fraction to 39% and improved both global and regional wall motion indices. Cerebral GLP-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons in rats, leading to downstream activation of cardiovascular responses [26]. Furthermore, it has been suggested that catecholaminergic neurons in the area postrema expressing the GLP-1 receptor may link peripheral GLP-1 and central autonomic control sites that mediate the diverse neuroendocrine and autonomic actions of peripheral GLP-1 [27]. It should be noted, however, that peripheral administration of GLP-1 in humans is not associated with changes in blood pressure or heart rate [28].

Recent studies showed that intracerebroventricular administration of GLP-1 was associated with improved learning in rats and neuroprotective effects [29,30]. GLP-1 has been proposed as a new therapeutic agent for neurodegenerative diseases, including Alzheimer's disease [31•].

Actions of native GLP-1 in type 2 diabetes

These actions render GLP-1 highly attractive as a therapeutic agent, but an extremely rapid enzymatic degradation of the molecule makes it unsuitable for injection therapy. This metabolism, which is attributable to the actions of the ubiquitous enzyme dipeptidyl peptidase IV (DPP-IV), results in a half-life for GLP-1 of only about two minutes [32]; furthermore, the actions on metabolism of single subcutaneous injections are short-lived. However, continuous subcutaneous infusion using insulin pumps was employed in a study where the hormone was given for six weeks to probe its effects in patients with T2DM [28]. Patients were evaluated before, after one week and after six weeks of treatment. No changes were observed in the saline-treated group, whereas in the GLP-1 group fasting and average plasma glucose concentrations were lowered by approximately 5 mmol/l; glycated haemoglobin (HbA1c; a long-term [months] measure of mean plasma glucose concentrations) decreased by 1.2%; free fatty acids were significantly lowered; and the patients had a gradual weight loss of approximately 2 kg. In addition, insulin sensitivity (as determined by a hyperinsulinaemic euglycaemic clamp) almost doubled, and insulin secretion capacity (measured using a 30 mmol/l glucose clamp + arginine) greatly improved. There was no significant difference between results obtained after one and six weeks of treatment, but there was a tendency towards further improvement in plasma glucose as well as insulin secretion. There were few side effects and no differences between saline- and GLP-1-treated patients in this respect. Of note, the dose selected was not necessarily maximal (and was not associated with side effects). Further studies using the same technique indicated that a higher infusion rate might be even more effective [33].

The conclusion drawn was that GLP-1-based therapy has unusually attractive potential in diabetes treatment. Therefore, two strategies have been pursued: the development of DPP-IV-resistant analogues of GLP-1 and development of inhibitors of DPP-IV.

Resistant analogues or activators of the GLP-1 receptor

Exendin 4

DPP-IV cleaves peptides at the penultimate N-terminal amino acid residue if this is Pro or Ala (Ala in GLP-1). Therefore, substitution of this residue can render the molecule resistant [34]. However, this only prolongs the half-life of the molecule from 2 min to 4–5 min, because renal extraction and degradation effectively clears the plasma of substituted, as well as unsubstituted, GLP-1 [35]. A prolonged effect therefore requires changes that decrease renal elimination. Exendin 4, isolated from the saliva of the lizard *Heloderma suspectum* (also called the Gila monster) is such a molecule. It is 53% homologous to GLP-1 (but is not the GLP-1 of the Gila monster) and

is cleared from plasma at a rate of 1.8 ml/kg/min, which is similar in magnitude to the normal glomerular filtration rate [36]. Otherwise, exendin 4 appears to act in humans in a manner identical to that of GLP-1 [36]. The clinical usefulness of exendin 4 was evaluated in a proof-of-concept Phase II study recently reported by the Amylin Corporation [37]. Exendin 4 (now named AC2993 or Exenatide) was injected subcutaneously twice or three times daily for four weeks in patients already treated with metformin, SU or both. In all groups, there was a reduction in HbA1c ranging from 0.7% to 1.1%. The most common adverse effect was transient mild to moderate nausea. Mild hypoglycaemia was reported in about a third of the patients also treated with SU. This finding was not substantiated by measurements, but could reflect a partial uncoupling of the glucose dependency of the insulinotropic actions of GLP-1 by SU (as discussed above) but, conversely, also illustrates the potency of this combination. In late 2003, the company completed Phase III studies with a similar design, in which Exenatide was given as twice-daily injections initially in doses of 5 μg for one month, and subsequently at 5 μg or 10 μg per injection for five months. This approach reduced the tendency to cause initial nausea. Mild hypoglycemia was noted in 35% of the patients also treated with SU. The average drop in HbA1c over six months of treatment was 1% from a base line value >8%, and values below 7% (the currently recommended target) were observed in 40–46% of patients (at 10 μg). Antibodies against Exenatide were observed in approximately one-fifth of the patients, but this was unrelated to the clinical efficacy. Recent studies presented by the Amylin Corporation at the American Diabetes Association (ADA) in Orlando 2004 indicated that subcutaneous injections of a stable GLP-1 receptor agonist (exendin 4) twice-daily for a year to individuals with T2DM were associated with a gradual weight loss, with no signs of impaired efficacy over time. Indeed, the efficacy of this appetite-reducing effect was demonstrated convincingly not only in these clinical studies but also in recent studies involving lifetime administration of exendin 4 to rats. The treated animals survived longer than controls, an effect that was thought to result from decreased food intake and hence a significantly lower body weight [38].

It can be concluded that Exenatide provides considerable additional glycemic control, even in patients inadequately treated with oral antidiabetic agents, and also causes weight loss, which can be predicted to provide further improvements of metabolism. However, two injections of exenatide per day do not provide a full 24-hour exposure to the GLP-1 receptor agonist, which is considered important for the full anti-diabetic effect of intravenously administered GLP-1 [39]; this might explain a less conspicuous effect on fasting plasma glucose.

Albumin-bound GLP-1 derivatives

Another approach has been to bind a GLP-1 analogue to albumin to exploit the slow elimination kinetics of this molecule in the body. Three different methods have been employed to achieve this: NovoNordisk in Denmark developed an acylated derivative of GLP-1 that binds non-covalently to albumin; the Canadian company, Conjuchem, created an analogue of GLP-1 which, after injection, establishes a covalent bond with albumin; and the American company Human Genome Sciences has generated a fusion protein consisting of a DPP-IV-resistant GLP-1 analogue covalently bound to human albumin.

NN2211

The selection of the NovoNordisk compound NN2211 for clinical development was recently described [40]. It consists of native GLP-1, in which a C16 acyl chain is attached via a glutamoyl spacer to Lys26 (Lys34 was substituted by Arg). The compound shows a slow release from the subcutaneous injection site and binds to albumin, which renders the molecule resistant to DPP-IV and allows at least the bound fraction to escapes renal elimination. This resulted in a half-life in healthy subjects and patients with T2DM of 10–12 h following a single subcutaneous injection [41] and thereby adequate 24 h exposure after a single daily injection. In addition, in chronic treatment, the large post-injection concentration excursions caused by less long-lived analogues that might be associated with side effects such as nausea are likely to be avoided, as shown in studies in pigs [42]. The analogue itself is equipotent to GLP-1 at the cloned human GLP-1 receptor. In clinical studies, NN2211 effectively reduced fasting, as well as meal-related (12 h post-injection) glycaemia, by modifying insulin secretion, delaying gastric emptying and suppressing prandial glucagon secretion [41] and, in a one-week study, improved both α and β cell function and reduced hepatic glucose production [43]. Phase II studies involving three months of daily injections have recently been reported [44]. In a blinded design, ascending doses of NN2211 in monotherapy were compared with glimepiride. It was concluded that NN2211 improved glycaemic control significantly and was comparable to glimepiride. Weight was maintained with a tendency to decrease, and the risk of hypoglycemica was low. It is noteworthy that antibodies against NN2211 could not be detected. This analogue clearly possesses favorable pharmacokinetic properties.

CJC-1131

The Conjuchem compound CJC-1131 is composed of a D-Ala8-substituted GLP-1 molecule with a linker and a reactive moiety (maleimidoproprionic acid) attached to the C-terminus. After injection *in vivo*, this molecule conjugates covalently to Lys34 of the albumin molecule and thereby acquires the half-life of albumin. The CJC-1131–albumin conjugate binds to the GLP-1 receptor and

activates cAMP with a potency similar to that of GLP-1 [45]. In recent studies in human volunteers, elimination half-lives ranging from 9–14 days were noted [46], and in studies presented at the International Diabetes Federation Congress in 2003 the compound was reported to have dose-dependent effects on glycaemia and body weight lasting at least 48 h, and up to eight days in some patients. Results of Phase II clinical studies have recently (July 2004) been announced as a press release from the company and, although effective in reduced fasting blood glucose and HbA1c levels as well as body weight, the compound was most effective when administered once daily, contrasting with the reported long half-life. The company claims that the conjugate is not antigenic.

Albugon

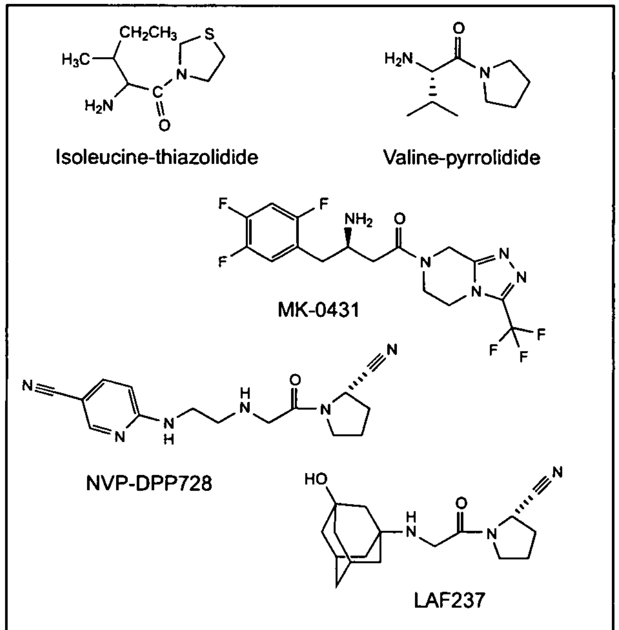
Very little is known about the Albugon compound from Human Genome Sciences. However, it has been reported to retain the insulinotropic activities of GLP-1 and to delay gastric emptying. In glucose-intolerant mice and in diabetic rats, a single injection almost normalized glucose levels for 24 h; the half life was said to be three days in monkeys [47].

Inhibitors of DPP-IV

Preclinical studies

Therapeutic use of inhibitors of the enzyme responsible for the inactivation of GLP-1 as anti-diabetic agents was first proposed in 1995 [48] on the basis of the finding that GLP-1 seems uniquely sensitive to cleavage by DPP IV; compounds of this class have now reached Phase III clinical trials. With a DPP-IV inhibitor (see Figure 1), it is possible to completely prevent the N-terminal degradation of GLP-1 that occurs *in vivo*, resulting in significant enhancement of its insulinotropic activity [49]. Studies in Vancouver diabetic fatty rats have shown that chronic oral administration of the Probiodrug DPP-IV inhibitor isoleucine thiazolidide (P32/98) for 12 weeks improves glucose tolerance, insulin sensitivity and β-cell responsiveness [50]. The longer-acting Ferring inhibitor, FE 999-011, continuously inhibits plasma DPP IV activity and not only normalises the glucose excursion after oral glucose administration in insulin-resistant Zucker obeses rats but also delays the onset of hyperglycaemia in Zucker diabetic fatty rats [51]. These effects were, at least in part, attributed to increased levels of intact GLP-1. Increased intact GLP-1 concentrations were also implicated in the improved islet function seen after chronic treatment of high-fat fed (glucose-intolerant and insulin-resistant) mice with valine-pyrrolidide [52]. Fischer rats, which have a catalytically inactive DPP-IV molecule, and CD26 knockout mice with a targeted disruption of the gene encoding DPP-IV further support the involvement of DPP-IV in mediating glucose tolerance. Such animals have improved glucose tolerance compared with their wild-type counterparts [53–55]. In DPP-IV-negative Fischer rats and DPP-IV inhibitor-treated control animals, the impaired glucose tolerance that normally develops with ageing is prevented [55,56], whereas the lack of DPP-IV protects both Fischer rats and CD26 knockout mice from diet (high fat)-induced insulin resistance and glucose intolerance [56–58]. Again, these effects are believed to involve preservation of endogenous GLP-1 levels, because intact GLP-1 concentrations are elevated.

Figure 1



Structures of DPP-IV inhibitors.

Clinical studies

After these promising preclinical studies, the first clinical proof-of-concept was obtained using the short-acting Novartis inhibitor, NVP-DPP728 [59]. When given twice or three times daily for four weeks in patients with relatively mild T2DM (mean HbA1c of 7.4%), both fasting and prandial glucose levels were lowered significantly, resulting in a reduction in HbA1c of 0.5%; despite the fall in glycemia, fasting and post-prandial insulin levels were sustained. NVP-DPP728 appeared to be well tolerated, with only minor adverse events being reported. However, some of these symptoms (pruritus and nasopharyngitis) seem to be drug- rather than class-specific, because they were not reported for another inhibitor, LAF237, also developed by Novartis. NVP-DPP728 has now been dropped in favour of LAF237, which is longer-acting and suitable for once-daily administration. A clinical study with this compound was recently reported, showing it to have a pharmacodynamic profile similar to that of its predecessor [60]. The mechanism of

action was suggested to be incretin-mediated, because LAF237 treatment increased both baseline and prandial active GLP-1 levels. As with NVP-DPP78, insulin levels were not increased but, interestingly, glucagon levels were significantly suppressed. Clinical data from longer-term studies presented at the recent ADA meeting in Orlando showed that 12 weeks of monotherapy with LAF237 was associated with sustained reductions in HbA1c (from a starting level of 8%, falling to 7.4% at the end of the study) [61]. Encouragingly, patients with the worst metabolic control (HbA1c ranging between 8% and 9.5%) showed the greatest reductions (1.2%), suggesting that DPP-IV inhibition may not be restricted only to those patients with mild diabetes. Furthermore, LAF237 was able to prevent the worsening of glycaemic control when given for up to 12 months in combination with metformin in patients otherwise inadequately controlled with metformin alone [62]. Side effects were mild and, importantly, hypoglycemia was not reported. However, in contrast to GLP-1 analogues, there was no change in body weight. Phase III clinical trials are currently in progress, and filing for FDA approval is expected in 2006.

Merck also has an inhibitor (MK-0431) in Phase III trials (http://www.merck.com), but so far little is known about this compound. Results of placebo-controlled, single-dose studies were presented at the ADA in Orlando. MK-0431 was well tolerated and caused significant reductions in the glycaemic excursion following an oral glucose tolerance test, which were associated with increases in intact GLP-1 and insulin, and reductions in glucagon secretion [63].

DPP-IV inhibitors are in development at GlaxoSmithKline (Phase I), Bristol-Meyer-Squibb (Phase II) and Probiodrug (P93/01; Phase II), with several other companies reportedly having a DPP-IV inhibitor programme. Single doses of P93/01 were shown to have good tolerability and result in dose-related reductions in prandial glucose in T2DM subjects when HbA1c was above 6% [64].

The clinical studies with DPP IV inhibitors that have been reported so far have not been associated with any serious adverse side effects, but there has been understandable concern that undesirable side effects could arise from inhibiting an enzyme with multiple substrates or because of non-mechanism-based actions (i.e. not related to the selective inhibition of DPP-IV). With regard to multiple substrates, although several regulatory peptides, neuropeptides, chemokines and cytokines have been identified as potential substrates from *in vitro* kinetic studies (reviewed by Lambeir *et al.* [65•]), it is uncertain how many of these are endogenous substrates and, if so, whether DPP-IV-mediated degradation is their primary route of elimination. In addition to GLP-1, the other incretin hormone, glucose-dependent insulinotropic polypeptide, is an endogenous DPP-IV substrate, as is the neuropeptide pituitary adenylate cyclase-activating peptide [66], but inhibition of their degradation would be expected to contribute to the anti-diabetic effects of DPP-IV inhibitors. The evidence for a physiological role for DPP-IV in degradation of many of the other potential substrates remains to be demonstrated. DPP-IV also has several other roles that could potentially be compromised by DPP-IV inhibition. It is present on the surface of T cells (where it is usually referred to as the T cell marker CD26) and contributes to T cell activation and proliferation via its interaction with other membrane-expressed molecules such as CD45, although it is uncertain whether the catalytic activity is required, or indeed whether its presence is obligatory [67]. In this context, a family of DPP-IV-related enzymes is now known to exist, which have similar catalytic activities. Selective inhibition of two of these enzymes (DPP 8 and DPP 9) was recently reported to affect T cell activation *in vitro* [68] and be associated with severe, even lethal side effects in preclinical species [69], whereas selective DPP-IV inhibition was not, suggesting that DPP 8 and 9 could be responsible for some of the functions previously attributed to DPP-IV. In turn, this raises the possibility that some of the potential or reported side effects of DPP IV inhibition could be attributable to inhibition of DPP 8 and 9, rather than DPP-IV itself. It is, therefore, highly relevant that rodents which lack DPP-IV enzymatic activity (the Fischer rat and the CD26 knockout mouse) are completely viable and seem to suffer no ill effects because of the lack of DPP-IV. Selectivity data for the inhibitors in development have not been released, apart from the Merck compound, which is reported to have >2500-fold selectivity for DPP-IV relative to DPP 8 and 9 [70].

Conclusions

It seems clear from the most recent clinical results that both GLP-1 analogues (or GLP-1 receptor activators such as exendin) and DPP-IV inhibitors effectively improve metabolic control in patients with T2DM. Both seem to be effective in monotherapy and in combination with other antidiabetic agents. DDP-IV inhibitors are administered orally, whereas the analogues require parenteral administration. The analogues cause significant reductions in body weight, whereas the inhibitors seem to be weight neutral. Clinical data obtained so far do not allow conclusions to be drawn on whether the protective effect on β-cells seen in laboratory animals can also be demonstrated in patients with T2DM.

Acknowledgements

The authors were supported by grants from The Danish Medical Research Council and the European Foundation for the Study of Diabetes.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Holst JJ, Gromada J: **Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans.** *Am J Physiol Endocrinol Metab* 2004, **287**:E199-E206.

2. Nauck M, Stockmann F, Ebert R, Creutzfeldt W: **Reduced incretin effect in type 2 (non-insulin-dependent) diabetes.** *Diabetologia* 1986, **29**:46-52.

3. Vilsboll T, Holst JJ: **Incretins, insulin secretion and type 2 diabetes mellitus.** *Diabetologia* 2004, **47**:357-366.

4. Kjems LL, Holst JJ, Volund A, Madsbad S: **The influence of GLP-1 on glucose-stimulated insulin secretion: effects on beta-cell sensitivity in type 2 and nondiabetic subjects.** *Diabetes* 2003, **52**:380-386.

5. Vilsboll T, Knop FK, Krarup T, Johansen A, Madsbad S, Larsen S, Hansen T, Pedersen O, Holst JJ: **The pathophysiology of diabetes involves a defective amplification of the late-phase insulin response to glucose by glucose-dependent insulinotropic polypeptide-regardless of etiology and phenotype.** *J Clin Endocrinol Metab* 2003, **88**:4897-4903.

6. Rachman J, Barrow BA, Levy JC, Turner RC: **Near-normalisation of diurnal glucose concentrations by continuous administration of glucagon-like peptide-1 (GLP-1) in subjects with NIDDM.** *Diabetologia* 1997, **40**:205-211.

7. Gutniak M, Orskov C, Holst JJ, Ahren B, Efendic S: **Antidiabetogenic effect of glucagon-like peptide-1 (7-36)amide in normal subjects and patients with diabetes mellitus.** *N Engl J Med* 1992, **326**:1316-1322.

8. Holst JJ: **Therapy of type 2 diabetes mellitus based on the actions of glucagon- like peptide-1.** *Diabetes Metab Res Rev* 2002, **18**:430-441.

9. Fehmann HC, Habener JF: **Insulinotropic hormone glucagon-like peptide-I(7-37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma beta TC-1 cells.** *Endocrinology* 1992, **130**:159-166.

10. Buteau J, Roduit R, Susini S, Prentki M: **Glucagon-like peptide-1 promotes DNA synthesis, activates phosphatidylinositol 3-kinase and increases transcription factor pancreatic and duodenal homeobox gene 1 (PDX-1) DNA binding activity in beta (INS-1)-cells.** *Diabetologia* 1999, 42:856-864.

11. Egan JM, Bulotta A, Hui H, Perfetti R: **GLP-1 receptor agonists are growth and differentiation factors for pancreatic islet beta cells.** *Diabetes Metab Res Rev* 2003, **19**:115-123.

12. Xu G, Stoffers DA, Habener JF, Bonner-Weir S: **Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats.** *Diabetes* 1999, **48**:2270-2276.

13. Stoffers DA, Kieffer TJ, Hussain MA, Drucker DJ, Bonner-Weir S, Habener JF, Egan JM: **Insulinotropic glucagon-like peptide 1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in mouse pancreas.** *Diabetes* 2000, **49**:741-748.

14. Zhou J, Wang X, Pineyro MA, Egan JM: **Glucagon-like peptide 1 and exendin-4 convert pancrteatic AR42J cells into glucagon- and insulin-producing cells.** *Diabetes* 1999, **48**:2358-2366.

15. Perfetti R, Zhou J, Doyle ME, Egan JM: **Glucagon-like peptide-1 induces cell proliferation and pancreatic- duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats.** *Endocrinology* 2000, **141**:4600-4605.

16. •• Buteau J, El-Assaad W, Rhodes CJ, Rosenberg L, Joly E, Prentki M: **Glucagon-like peptide-1 prevents beta cell glucolipotoxicity.** *Diabetologia* 2004, **47**:806-815.
Demonstrates the powerful protective effects of GLP-1 on apoptosis induced by lipo- or gluco-toxicity in human β-cells.

17. Toft-Nielsen MB, Damholt MB, Madsbad S, Hilsted LM, Hughes TE, Michelsen BK, Holst JJ: **Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients.** *J Clin Endocrinol Metab* 2001, **86**:3717-3723.

18. Wettergren A, Schjoldager B, Mortensen PE, Myhre J, Christiansen J, Holst JJ: **Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man.** *Dig Dis Sci* 1993, **38**:665-673.

19. Nauck MA, Niedereichholz U, Ettler R, Holst JJ, Orskov C, Ritzel R, Schmiegel WH: **Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans.** *Am J Physiol* 1997, **273**:E981-E988.

20. Young A, Denaro M: **Roles of amylin in diabetes and in regulation of nutrient load.** *Nutrition* 1998, **14**:524-527.

21. Verdich C, Flint A, Gutzwiller JP, Naslund E, Beglinger C, Hellstrom PM, Long SJ, Morgan LM, Holst JJ, Astrup A: **A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on ad libitum energy intake in humans.** *J Clin Endocrinol Metab* 2001, **86**:4382-4389.

22. Bullock BP, Heller RS, Habener JF: **Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor.** *Endocrinology* 1996, **137**:2968-2978.

23. • Gros R, You X, Baggio LL, Kabir MG, Sadi AM, Mungrue IN, Parker TG, Huang Q, Drucker DJ, Husain M: **Cardiac function in mice lacking the glucagon-like peptide-1 receptor.** *Endocrinology* 2003, **144**:2242-2252.
Demonstrates that heart function is impaired in the absence of GLP-1.

24. •• Bose KA, Mocanu MM, Carr RD, Brand CL, Yellon DM: **Glucagon-like peptide-1(GLP-1) can directly protect the heart against ischemia/reperfusion injury.** *Diabetes* 2004, in press.
Demonstrates clinical improvement of cardiac function with GLP-1 in patients with cardiac failure after myocardial infarction and angioplasty.

25. Nikolaidis LA, Mankad S, Sokos GG, Miske G, Shah A, Elahi D, Shannon RP: **Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion.** *Circulation* 2004, **109**:962-965.

26. Yamamoto H, Lee CE, Marcus JN, Williams TD, Overton JM, Lopez ME, Hollenberg AN, Baggio L, Saper CB, Drucker DJ, Elmquist JK: **Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons.** *J Clin Invest* 2002, **110**:43-52.

27. Yamamoto H, Kishi T, Lee CE, Choi BJ, Fang H, Hollenberg AN, Drucker DJ, Elmquist JK: **Glucagon-like peptide-1-responsive catecholamine neurons in the area postrema link peripheral glucagon-like peptide-1 with central autonomic control sites.** *J Neurosci* 2003, **23**:2939-2946.

28. Zander M, Madsbad S, Madsen JL, Holst JJ: **Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study.** *Lancet* 2002, **359**:824-830.

29. Perry T, Haughey NJ, Mattson MP, Egan JM, Greig NH: **Protection and reversal of excitotoxic neuronal damage by glucagon-like peptide-1 and exendin-4.** *J Pharmacol Exp Ther* 2002, **302**:881-888.

30. During MJ, Cao L, Zuzga DS, Francis JS, Fitzsimons HL, Jiao X, Bland RJ, Klugmann M, Banks WA, Drucker DJ, Haile CN: **Glucagon-like peptide-1 receptor is involved in learning and neuroprotection.** *Nat Med* 2003, **9**:1173-1179.

31. • Perry TA, Greig NH: **A new Alzheimer's disease interventive strategy: GLP-1.** *Curr Drug Targets* 2004, **5**:565-571.
This review summarizes the evidence that GLP-1 might also have neuroprotective effects.

32. Vilsboll T, Agerso H, Krarup T, Holst JJ: **Similar elimination rates of glucagon-like peptide-1 in obese type 2 diabetic patients and healthy subjects.** *J Clin Endocrinol Metab* 2003, **88**:220-224.

33. Ehlers MRW, Roderick EH, Schneider RL, Kipnes MS: **Continuous subcutaneous infusion of recombinant GLP-1 for 7 days dose-dependently improved glycemic controls in type 2 diabetes [abstract].** *Diabetes* 2002, **51 (Suppl 2)**:A579.

34. Deacon CF, Knudsen LB, Madsen K, Wiberg FC, Jacobsen O, Holst JJ: **Dipeptidyl peptidase IV resistant analogues of glucagon-like peptide-1 which have extended metabolic stability and improved biological activity.** *Diabetologia* 1998, **41**:271-278.

35. Meier JJ, Nauck MA, Kranz D, Holst JJ, Deacon CF, Gaeckler D, Schmidt WE, Gallwitz B: **Secretion, degradation, and elimination of glucagon-like peptide 1 and gastric inhibitory polypeptide in patients with chronic renal insufficiency and healthy control subjects.** *Diabetes* 2004, **53**:654-662.

36. Edwards CM, Stanley SA, Davis R, Brynes AE, Frost GS, Seal LJ, Ghatei MA, Bloom SR: **Exendin-4 reduces fasting and postprandial glucose and decreases energy intake in healthy volunteers.** *Am J Physiol Endocrinol Metab* 2001, **281**:E155-E161.

37. Fineman MS, Bicsak TA, Shen LZ, Taylor K, Gaines E, Varns A, Kim D, Baron AD: **Effect on glycemic control of exenatide (synthetic exendin-4) additive to existing metformin and/or sulfonylurea treatment in patients with type 2 diabetes.** *Diabetes Care* 2003, **26**:2370-2377.

38. Hiles R, Carpenter T, Serota D, Schafer K, Ross P, Nelsen D, Rebelatto M: **Exenatide does not cause pancreatic tumours or malignancies in rats and mice following a 2-year period of exposure [abstract].** *Diabetes* 2004, **53 (Suppl 2)**:A380.

39. Larsen J, Hylleberg B, Ng K, Damsbo P: **Glucagon-like peptide-1 infusion must be maintained for 24 h/day to obtain acceptable glycemia in type 2 diabetic patients who are poorly controlled on sulphonylurea treatment.** *Diabetes Care* 2001, **24**:1416-1421.

40. Knudsen LB, Agersø H, Bjenning C, Bregenholt S, Gotfredsen C, Holst JJ, Huusfeldt PO, Larsen MØ, Larsen PJ, Nielsen PF *et al.*: **GLP-1 derivatives as novel compounds for the treatment of type 2 diabetes.** *Drugs of the Future* 2001, **26**:677-685.

41. Juhl CB, Hollingdal M, Sturis J, Jakobsen G, Agerso H, Veldhuis J, Porksen N, Schmitz O: **Bedtime administration of NN2211, a long-acting GLP-1 derivative, substantially reduces fasting and postprandial glycemia in type 2 diabetes.** *Diabetes* 2002, **51**:424-429.

42. Ribel U, Larsen MO, Rolin B, Carr RD, Wilken M, Sturis J, Westergaard L, Deacon CF, Knudsen LB: **NN2211: a long-acting glucagon-like peptide-1 derivative with anti-diabetic effects in glucose-intolerant pigs.** *Eur J Pharmacol* 2002, **451**:217-225.

43. Degn KB, Juhl CB, Sturis J, Jakobsen G, Brock B, Chandramouli V, Rungby J, Landau BR, Schmitz O: **One week's treatment with the long-acting glucagon-like peptide 1 derivative liraglutide (NN2211) markedly improves 24-h glycemia and alpha- and beta-cell function and reduces endogenous glucose release in patients with type 2 diabetes.** *Diabetes* 2004, **53**:1187-1194.

44. Madsbad S, Schmitz O, Ranstam J, Jakobsen G, Matthews DR: **Improved glycemic control with no weight increase in patients with type 2 diabetes after once-daily treatment with the long-acting glucagon-like peptide 1 analog liraglutide (NN2211): a 12-week, double-blind, randomized, controlled trial.** *Diabetes Care* 2004, **27**:1335-1342.

45. Kim JG, Baggio LL, Bridon DP, Castaigne JP, Robitaille MF, Jette L, Benquet C, Drucker DJ: **Development and characterization of a glucagon-like peptide 1-albumin conjugate: the ability to activate the glucagon-like peptide 1 receptor *in vivo*.** *Diabetes* 2003, **52**:751-759.

46. Lawrence B, Dreyfus J, Wen S, Guicarc'h P, Drucker D, Castaigne JP: **CJC-II31, a long acting GLP-1 derivative, exhibits an extended pharmacokinetic profile in healthy human volunteers [abstract].** *Diabetes* 2003, **52 (Suppl 1)**:A125.

47. Bloom M, Bock J, Duttaroy A, Grzegorzewski K, Moor P, Ou Y, Wojcik S, Zhou X, Bell A: **Albugon fusion protein: A long acting analogue of GLP-1 that provides lasting antidiabetic effect in animals [abstract].** *Diabetes* 2003, **52 (Suppl 1)**:A112.

48. Deacon CF, Nauck MA, Toft-Nielsen M, Pridal L, Willms B, Holst JJ: **Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH2-terminus in type II diabetic patients and in healthy subjects.** *Diabetes* 1995, **44**:1126-1131.

49. Deacon CF, Hughes TE, Holst JJ: **Dipeptidyl peptidase IV inhibition potentiates the insulinotropic effect of glucagon-like peptide 1 in the anesthetized pig.** *Diabetes* 1998, **47**:764-769.

50. Pospisilik JA, Stafford SG, Demuth HU, Brownsey R, Parkhouse W, Finegood DT, McIntosh CH, Pederson RA: **Long-term treatment with the dipeptidyl peptidase IV inhibitor P32/98 causes sustained improvements in glucose tolerance, insulin sensitivity, hyperinsulinemia, and beta-cell glucose responsiveness in VDF (fa/fa) Zucker rats.** *Diabetes* 2002, **51**:943-950.

51. Sudre B, Broqua P, White RB, Ashworth D, Evans DM, Haigh R, Junien JL, Aubert ML: **Chronic inhibition of circulating dipeptidyl peptidase IV by FE 999011 delays the occurrence of diabetes in male zucker diabetic fatty rats.** *Diabetes* 2002, **51**:1461-1469.

52. Reimer MK, Holst JJ, Ahren B: **Long-term inhibition of dipeptidyl peptidase IV improves glucose tolerance and preserves islet function in mice.** *Eur J Endocrinol* 2002, **146**:717-727.

53. Nagakura T, Yasuda N, Yamazaki K, Ikuta H, Yoshikawa S, Asano O, Tanaka I: **Improved glucose tolerance via enhanced glucose-dependent insulin secretion in dipeptidyl peptidase IV-deficient Fischer rats.** *Biochem Biophys Res Commun* 2001, **284**:501-506.

54. Marguet D, Baggio L, Kobayashi T, Bernard AM, Pierres M, Nielsen PF, Ribel U, Watanabe T, Drucker DJ, Wagtmann N: **Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26.** *Proc Natl Acad Sci USA* 2000, **97**:6874-6879.

55. Mitani H, Takimoto M, Kimura M: **Dipeptidyl peptidase IV inhibitor NVP-DPP728 ameliorates early insulin response and glucose tolerance in aged rats but not in aged Fischer 344 rats lacking its enzyme activity.** *Jpn J Pharmacol* 2002, **88**:451-458.

56. Mitani H, Takimoto M, Hughes TE, Kimura M: **Dipeptidyl peptidase IV inhibition improves impaired glucose tolerance in high-fat diet-fed rats: study using a Fischer 344 rat substrain deficient in its enzyme activity.** *Jpn J Pharmacol* 2002, **88**:442-450.

57. Yasuda N, Nagakura T, Yamazaki K, Inoue T, Tanaka I: **Improvement of high fat-diet-induced insulin resistance in dipeptidyl peptidase IV-deficient Fischer rats.** *Life Sci* 2002, **71**:227-238.

58. Conarello SL, Li Z, Ronan J, Roy RS, Zhu L, Jiang G, Liu F, Woods J, Zycband E, Moller DE *et al.*: **Mice lacking dipeptidyl peptidase IV are protected against obesity and insulin resistance.** *Proc Natl Acad Sci USA* 2003, **100**:6825-6830.

59. Ahren B, Simonsson E, Larsson H, Landin-Olsson M, Torgeirsson H, Jansson PA, Sandqvist M, Bavenholm P, Efendic S, Eriksson JW *et al.*: **Inhibition of dipeptidyl peptidase IV improves metabolic control over a 4-week study period in type 2 diabetes.** *Diabetes Care* 2002, **25**:869-875.

60. Ahren B, Landin-Olsson M, Jansson PA, Svensson M, Holmes D, Schweizer A: **Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes.** *J Clin Endocrinol Metab* 2004, **89**:2078-2084.

61. Pratley RE, Galbreath E: **Twelve-week monotherapy qwith the DPP-4 inhibitor, LAF 237 improves glycemic control in patients with type 2 diabetes (T2DM) [abstract].** *Diabetes* 2004, **53 (Suppl 2)**:A83.

62. Ahren B, Gomis R, Standl E, Mills D, Schweizer A: **Prolonged efficacy of LAF237 in patients with type 2 diabetes (T2DM) inadequately treated with metformin [abstract].** *64th Scientific Sessions of the American Diabetes Association 2004. Orlando, USA.*

63. Herman GA, Zhao PL, Dietrich B *et al.*: **The DP-IV inhibitor MK-0431 enhances active GLP-1 and reduces glucose following an OGTT in type 2 diabetics [abstract].** *Diabetes* 2004, **53(Suppl 2)**:A82.

64. Heins J, Glund K, Hoffmann T, Metzner J, Demuth H-U: **The DP-IV inhibitor P93/01 improves glucose tolerance in humans with HbA1c gerater than 6.0 [abstract].** *Diabetes* 2004, **53(Suppl 2)**:A128.

65. Lambeir AM, Durinx C, Scharpe S, De Meester I:
• **Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions, and clinical aspects of the enzyme DPP IV.** *Crit Rev Clin Lab Sci* 2003, **40**:209-294.
A very useful and comprehensive review of the enzymology of DPP-IV.

66. Zhu L, Tamvakopoulos C, Xie D, Dragovic J, Shen X, Fenyk-Melody JE, Schmidt K, Bagchi A, Griffin PR, Thornberry NA, Sinha RR: **The role of dipeptidyl peptidase IV in the cleavage of glucagon family peptides: *in vivo* metabolism of pituitary adenylate cyclase activating polypeptide-(1-38).** *J Biol Chem* 2003, **278**:22418-22423.

67. von Bonin A, Huhn J, Fleischer B: **Dipeptidyl-peptidase IV/CD26 on T cells: analysis of an alternative T-cell activation pathway.** *Immunol Rev* 1998, **161**:43-53.

68. Leiting B, Nichols E, Biftu T, Edmonson S, Ok H, Weber AE, Zaller D, Thornberry NA: **Inhibition of dipeptidyl peptidase IV does not attenuate T cell activation *in vitro* [abstract].** *Diabetes* 2004, **53 (Suppl 2)**:A2.

69. Lankas G, Leiting B, Roy RS, Eiermann G, Biftu T, Kim D, Ok H, Weber AE, Thornberry NA: **Inhibition of DPP8/9 results in toxicity in preclinical species: potential importance of selective dipeptidyl peptidase IV ionhibition for the treatment of type 2 DM (abstract].** *Diabetes* 2004, **53 (Suppl 2)**:A2.

70. Weber AE, Kim D, Beconi M *et al.*: **MK-0431 is a potent, selective dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes [abstract].** *Diabetes* 2004, **53 (Suppl 2)**:A151.

Therapeutic Strategies Based on Glucagon-Like Peptide 1

Carolyn F. Deacon

Glucagon-like peptide (GLP)-1 is an incretin hormone with potent glucose-dependent insulinotropic and glucagonostatic actions, trophic effects on the pancreatic β-cells, and inhibitory effects on gastrointestinal secretion and motility, which combine to lower plasma glucose and reduce glycemic excursions. Furthermore, via its ability to enhance satiety, GLP-1 reduces food intake, thereby limiting weight gain, and may even cause weight loss. Taken together, these actions give GLP-1 a unique profile, considered highly desirable for an antidiabetic agent, particularly since the glucose dependency of its antihyperglycemic effects should minimize any risk of severe hypoglycemia. However, its pharmacokinetic/pharmacodynamic profile is such that native GLP-1 is not therapeutically useful. Thus, while GLP-1 is most effective when administered continuously, single subcutaneous injections have short-lasting effects. GLP-1 is highly susceptible to enzymatic degradation in vivo, and cleavage by dipeptidyl peptidase IV (DPP-IV) is probably the most relevant, since this occurs rapidly and generates a noninsulinotropic metabolite. Strategies for harnessing GLP-1's therapeutic potential, based on an understanding of factors influencing its metabolic stability and pharmacokinetic/pharmacodynamic profile, have therefore been the focus of intense research in both academia and the pharmaceutical industry. Such strategies include DPP-IV–resistant GLP-1 analogs and selective enzyme inhibitors to prevent in vivo degradation of the peptide. *Diabetes* 53:2181–2189, 2004

When the gene encoding glucagon, the mammalian pancreatic hormone, was cloned, the structure of its precursor, proglucagon, was deduced and shown to contain the sequences of two additional peptides, named glucagon-like peptide (GLP)-1 and -2 because of their considerable sequence homology to glucagon (1). It was a further several years before two endogenous peptides, GLP-1 (7-36)amide (2) and GLP-1(7-37) (3) were identified. When these peptides were demonstrated to be highly potent insulinotropic agents (2–4), interest in GLP-1 research grew significantly.

GLP-1 possesses a number of properties that make it a potentially ideal antidiabetic agent. It is released from the intestinal L-cell in response to orally ingested nutrients and has effects on the endocrine pancreas, on the gastrointestinal tract, and in the brain (rev. in 5). Thus, in the pancreas, GLP-1 acts as an incretin hormone, stimulating meal-induced insulin secretion. This effect is glucose dependent, meaning that any risk of hypoglycemia during exogenous peptide administration is practically eliminated. GLP-1 not only stimulates insulin exocytosis, but it also promotes all steps in insulin biosynthesis (6). More recently, direct effects on β-cell growth and survival have been identified, with GLP-1–stimulated proliferation (7,8) and differentiation of new β-cells (9,10) leading to increased β-cell mass. There is also increasing evidence that GLP-1 receptor signaling results in a reduction of β-cell apoptosis (11–14), which will further contribute to increased β-cell mass. Moreover, GLP-1 inhibits glucagon secretion, which, notably, is also glucose dependent (15), meaning that GLP-1 administration is unlikely to impair the glucagon counterregulatory response to hypoglycemia. In the gastrointestinal tract, GLP-1 inhibits motility and secretion (16), thereby contributing to reduce the glucose excursion by delaying the passage of nutrients to the small intestine. Indeed, under physiological circumstances in healthy subjects, this effect appears to outweigh its insulinotropic effect (16). In humans, peripherally administered GLP-1 has a satiating effect (see 17), and when given over a prolonged period (6 weeks) by continuous subcutaneous infusion, patients with type 2 diabetes reported a reduction in appetite, which led to significant reductions in body weight (Fig. 1) by the end of the study (18). A decreased gastric emptying rate seems to be involved (17), but a reduced sensation of appetite during GLP-1 in the fasting state, before meal ingestion (19), suggests other mechanisms may also contribute. Central administration of GLP-1 inhibits food intake in rodents (20), raising the possibility that peripherally released GLP-1 may have direct effects on the brain, because circulating GLP-1 can access GLP-1 receptors in brain areas (subfornical organ, area postrema) that participate in the regulation of appetite and energy homeostasis (21). However, it is also relevant that gastric distension activates GLP-1–containing neurons in the caudal nucleus of the solitary tract, suggesting a role for centrally expressed GLP-1 as an

From the Department of Medical Physiology, The Panum Institute, Copenhagen, Denmark.

Address correspondence and reprint requests to Dr. C.F. Deacon, Department of Medical Physiology, Panum Institute, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark. E-mail: deacon@mfi.ku.dk.

Received for publication 1 April 2004 and accepted in revised form 27 May 2004.

C.F.D. is a paid consultant for Novo Nordisk.

DPP-IV, dipeptidyl peptidase IV; GIP, glucose-dependent insulinotropic polypeptide; GLP, glucagon-like peptide; NEP, neutral endopeptidase; OAA, oral antidiabetic agent.

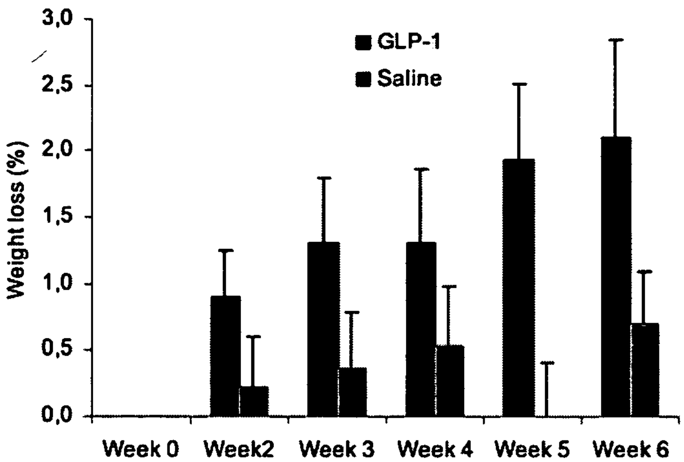


FIG. 1. Changes in body weight (expressed as a percentage of the pretreatment [week 0] weight) in type 2 diabetic patients assigned to continuous subcutaneous infusion of placebo (saline) or GLP-1 (4.8 pmol · kg^{-1} · min^{-1}) for 6 weeks. Treatment with GLP-1 for 6 weeks resulted in a significant ($P = 0.02$) reduction in body weight relative to pretreatment weight, whereas body weight after placebo treatment was not changed ($P = 0.4$). Reprinted with permission from Elsevier (Zander M, et al., *Lancet* 359:824–830, 2002).

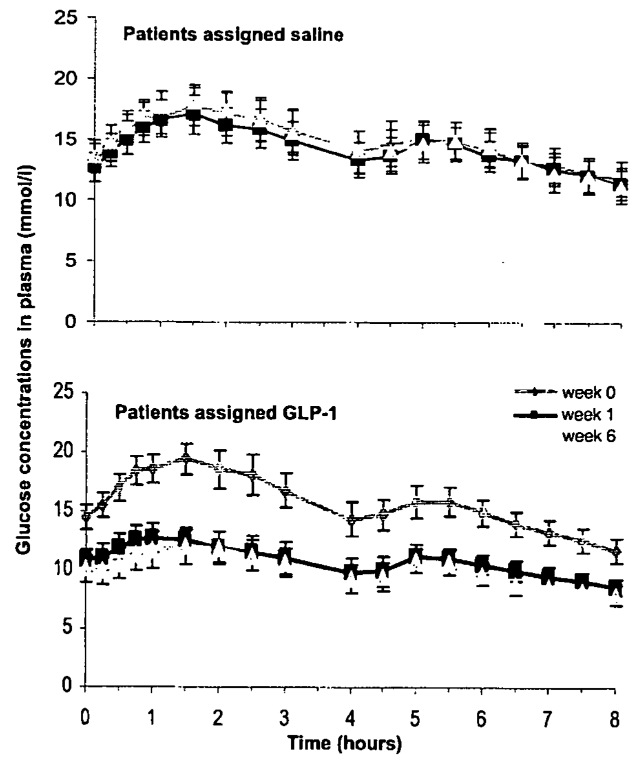


FIG. 2. Eight-hour plasma glucose profiles in type 2 diabetic patients assigned to continuous subcutaneous infusion of placebo (saline) or GLP-1 (4.8 pmol · kg^{-1} · min^{-1}) for 6 weeks, measured before treatment (week 0), and after 1 and 6 weeks of treatment. Treatment with GLP-1 led to significant reductions in fasting plasma glucose ($P < 0.0001$) and 8-h mean glucose concentrations ($P < 0.001$), but these parameters did not change significantly on placebo treatment ($P = 0.13$ and 0.95, respectively). Reprinted with permission from Elsevier (Zander M, et al., *Lancet* 359:824–830, 2002).

inhibitor of food intake (22). Interestingly, central administration of the GLP-1 receptor antagonist, exendin (9-39), increases food intake (20), suggesting that GLP-1 produced locally within the brain may exert a tonic satiating effect.

GLP-1 and diabetes. The incretin effect is known to be reduced in patients with type 2 diabetes, resulting in inappropriately low insulin secretion following oral ingestion of nutrients (23). More recent studies have indicated that GLP-1 secretion is also impaired in these subjects, suggesting that a reduced meal-related GLP-1 response may contribute to the decreased incretin effect (24). GLP-1 is effective in patients with type 2 diabetes, increasing insulin secretion and normalizing both fasting and postprandial blood glucose when given as a continuous intravenous infusion (25), even in subjects with advanced type 2 diabetes long after sulfonylurea secondary failure (26). Unexpectedly, the effects of a single subcutaneous injection of GLP-1 were disappointing. Although high plasma levels of immunoreactive GLP-1 were achieved, insulin secretion rapidly returned to pretreatment values and blood glucose concentrations were not normalized (27). Nevertheless, the effect of repeated subcutaneous administration on fasting blood glucose is as good as that of intravenous administration (27), while continuous subcutaneous administration for 6 weeks reduces fasting and postprandial glucose concentrations (Fig. 2) and lowers HbA_{1c} concentrations (18).

Incretin hormone metabolism. A possible explanation for the short-lived effectiveness of single subcutaneous injections of GLP-1 was indicated when it was shown that GLP-1 (and the other incretin, glucose-dependent insulinotropic polypeptide [GIP]) was metabolized by plasma in vitro and that the enzyme dipeptidyl peptidase-IV (DPP-IV) was capable of mediating this degradation (28). DPP-IV is a membrane-bound ectoenzyme, found in numerous sites, including the kidney, intestine, and capillary endothelium. It cleaves an NH_2-terminal dipeptide from peptides where the penultimate amino acid residue is proline or alanine (Fig. 3), and since these residues in GLP-1 (and GIP) are important for receptor activation, it was suggested that DPP-IV may be involved in regulating their biological activity (28). Indeed, pharmacological studies suggested that the GLP-1 metabolite [GLP-1 (9-36)amide] can behave as an antagonist at the pancreatic GLP-1 receptor (29), although subsequent in vivo studies demonstrated that it does not antagonize the insulinotropic effects of GLP-1 (30,31). Given the interest in developing an antidiabetic therapy based on GLP-1, these in vitro studies (28) spurred research into incretin hormone metabolism. Thus, Deacon et al. (32) reported that DPP-IV inhibition prevented GLP-1 degradation by human plasma in vitro and identified the truncated metabolite as an endogenous circulating peptide in humans, whereas Kieffer et al. (33) showed in vivo degradation of exogenous GLP-1 (and GIP) in normal rats, but not in a mutant strain lacking DPP-IV. Subsequently, exogenous GLP-1, particularly after subcutaneous injection, was demonstrated to be NH_2-terminally degraded in healthy and diabetic subjects (34). Taken together with the knowledge of DPP-IV's widespread distribution and the suggestion that the truncated metabolites may be unable to activate the respective incretin receptors (28), an important role for DPP-IV in the physiological regulation of GLP-1 and GIP activity was suggested (28,33,34). The finding that the enzyme is localized in the endothelium of capillaries actually adjacent to GLP-1–containing L-cells (35) and the demonstration that over half of newly synthe-

NEP 24.11

H-A-E-G-T-F-T-S-D-V-S-S-Y-L-E-G-Q-A-A-K-E-F-I-A-W-L-V-K-G-R-amide

DPP IV

FIG. 3. The amino acid sequence of GLP-1 (7-36)amide, showing the cleavage sites of DPP IV and NEP 24.11 (from 18 and 23).

sized (intact) GLP-1 is NH_2-terminally degraded even before it leaves the local capillary bed (35) further underscore the relevance of DPP-IV in incretin biology.

The involvement of a second enzyme in incretin hormone metabolism was suggested when GLP-1 was demonstrated to be a substrate for neutral endopeptidase (NEP) 24.11 in vitro (36,37). NEP 24.11 is a membrane-bound zinc metallopeptidase that cleaves peptides at the NH_2-terminal side of aromatic or hydrophobic amino acids, and six potential cleavage sites in GLP-1 were identified (Fig. 3) (36). GIP was also degraded by NEP 24.11, albeit more slowly, and it was suggested that its larger size (42 vs. 30 amino acids for GLP-1) may be one factor determining its suitability as a substrate, since the enzyme has a preference for smaller peptides (36), but the physiological significance was not examined in vivo. Since NEP 24.11 has a widespread tissue distribution and is found in particularly high concentration in the kidneys, it could be speculated to be involved in the renal clearance of peptide hormones.

Therapeutic strategies based on GLP-1. In our study showing that exogenous GLP-1 was rapidly NH_2-terminally degraded in both healthy and type 2 diabetic subjects, we discussed the potential physiological role of DPP-IV in incretin hormone metabolism and suggested that "inhibition of dipeptidyl peptidase IV may prove a useful adjunct in the management of type 2 diabetes" because "inhibition of GLP-1 (7-36)amide would . . . increase the availability of the biologically active peptide," and it was additionally suggested that DPP-IV–resistant GLP-1 analogs may also have therapeutic potential (34,38). Subsequently, with the confirmation of the pivotal role of DPP-IV and the postulated role of NEP 24.11 in incretin hormone metabolism, coupled with ongoing studies into the possibility of using GLP-1 therapeutically, many studies have addressed the possibility of manipulating the in vivo survival of GLP-1 as a novel approach to the treatment of diabetes. In this context, two separate approaches can be envisaged: *1*) the development of analogs of GLP-1 that are not susceptible to enzymatic degradation and *2*) the use of selective enzyme inhibitors to prevent in vivo degradation and enhance levels of the intact, biologically active peptides.

Enzyme-resistant GLP-1 analogs. This approach has been investigated experimentally, and promising compounds are now in the final stages of clinical development. Initial studies examined the effect of simply substituting the penultimate alanine in GLP-1 to render it more DPP-IV resistant. These analogs maintain their affinity for the GLP-1 receptor and are more stable in vivo (39,40), resulting in greater potency than native GLP-1 (40), but—although they are not degraded by DPP-IV—they are still cleared relatively quickly from the plasma by other mechanisms, meaning their usefulness in the clinical setting is likely to be limited.

Exendin-4 is a GLP-1 receptor agonist, originally isolated from the venom of the Gila monster, which shares 53% sequence homology with native GLP-1. It is resistant to DPP-IV (because of the penultimate NH_2-terminal glycine instead of alanine as in GLP-1) and survives longer in the circulation (plasma half-life of 26 min in humans [41] compared with 1–2 min for intact biologically active GLP-1 [42]). This may partly be due to exendin-4 being a poor substrate for NEP 24.11, because although the NH_2-terminal regions of both peptides show high sequence homology, several potential NEP 24.11 cleavage sites present in GLP-1 are absent in exendin-4 (36). In addition, by virtue of its COOH-terminal extension, exendin-4 is larger (39 amino acids) than GLP-1, which may contribute to it being a poorer substrate, because, as mentioned above, NEP 24.11 has a preference for smaller substrates. In contrast to GLP-1, which is cleared more rapidly, the metabolic clearance of exendin-4 in humans is similar to the glomerular filtration rate (41), suggesting that the kidneys are important in clearing exendin-4. In insulin-resistant diabetic mice, repeated administration of exendin-4 for 13 weeks increased plasma insulin and reduced blood glucose and HbA_{1c} concentrations (43). In Zucker rats, 8 weeks of exendin-4 treatment was associated with both reduced glycemia and insulin levels, suggesting improved glucose tolerance (44), and in addition, body weight gain was reduced. More recently, the effects of exendin-4 were examined in Goto-Kakizaki (GK) rats. In these animals, a genetic neonatal β-cell mass deficit is considered to be the primary defect leading to basal hyperglycemia and subsequent development of diabetes, but exendin-4 treatment during the first postnatal week (the pre-diabetic period) increases the β-cell mass, with subsequent improvements in glycemic control at adult age (45). In *db/db* mice, exendin-4, given in the pre-diabetic period, expands the functional β-cell mass via effects on both proliferation and apoptosis, delaying the development of diabetes (46), while neonatal GLP-1 or exendin-4 treatment stimulates β-cell neogenesis in newborn streptozotocin-injected rats (a model of β-cell regeneration), leading to both short- and long-term effects on β-cell mass recovery and glucose homeostasis (47). When given neonatally, exendin-4 prevents the subsequent development of diabetes in the intrauterine growth retarded rat by normalizing PDX (a pancreatic growth factor) levels and β-cell proliferation rates and preventing the progressive reduction in β-cell mass that usually occurs in this model (48). In healthy humans, acute intravenous infusions of exendin-4 are insulinotropic and reduce both fasting and postprandial glucose concentrations (41). Exenatide (AC2993, synthetic exendin-4) has now reached phase 3 of clinical development. In a placebo-controlled study in type 2 diabetic patients, exenatide reduces fasting glucose when given acutely, and postprandial glucose when given twice daily over 5 days before breakfast and dinner (49). However, in the 5-day study, there was no significant effect on pre-breakfast fasting glucose levels, suggesting that the duration of action of the previous evening's dose was

insufficient to maintain an antiglycemic effect overnight. This was confirmed in a 1-month study, where once-daily injections did not maintain satisfactory glucose control, but twice-daily treatment significantly improved HbA_{1c}, relative to pretreatment levels, even though full 24-h blood glucose control was still not achieved (50). When given in combination with ongoing oral antidiabetic agents (OAAs) (metformin and/or a sulfonylurea) two or three times daily, exenatide leads to further reductions in serum fructosamine and HbA_{1c} compared with OAAs alone (51). Preliminary findings from an ongoing clinical trial, in which twice-daily exenatide injections in addition to existing OAAs was compared to the baseline period with OAAs alone, indicate significant improvements in fasting plasma glucose and HbA_{1c} by 4 weeks that were maintained up to 20 weeks (52). Weight changes were not noted in the shorter-duration studies (50,51), but by the 20th week reductions in body weight were seen (52). There were some cases of hypoglycemia (15% overall), but notably only in patients also taking sulfonylureas, and none were reported as being severe (52). Some patients (19%) developed anti-exenatide antibodies, but these appeared not to influence glycemic control, and apart from mild/moderate nausea, no serious side effects were reported (51,52).

LY307161-SR is a sustained release formulation of a DPP-IV–resistant GLP-1 analog. Single daily injections of this compound for 12 weeks significantly improves both fasting and postprandial glucose concentrations in type 2 diabetic patients (53). However, many patients experienced adverse injection site reactions, leading to reduced compound exposure (53), and development has now been put on hold.

Another analog, liraglutide (NN2211; 97% homologous to native GLP-1), which is in late phase 2 of clinical development, has been designed to overcome both the effects of DPP-IV degradation and the short plasma survival time (54). Acylation with a fatty acid chain in liraglutide promotes binding to albumin, thereby reducing access to the NH_2-terminal by DPP-IV and allowing the molecule to escape renal filtration. Combined with delayed absorption from the injection site, this results in a stable analog with a plasma elimination half-life of around 12 h in humans, giving a pharmacodynamic profile suitable for once-daily dosing (55). Liraglutide reduces glycemia in insulin-resistant murine models of diabetes and is associated with increased β-cell mass and proliferation after 2 weeks of treatment (56). Similar findings were obtained in diabetic rats, in which β-cell mass changes correlated positively with the degree of hyperglycemia so that where normoglycemia was attained, the hyperglycemia-induced increase in β-cell mass was prevented (57). In acute (single-dose) placebo-controlled crossover clinical studies, liraglutide reduces fasting and postprandial glucose concentrations in type 2 diabetic patients (55) and is associated with restoration of β-cell responsiveness to physiological hyperglycemia (58). Results from longer-duration studies have only recently been reported. Thus, studies in type 2 diabetic patients indicated that 1-week treatment with once-daily liraglutide significantly reduces 24-h glucose concentrations and improves β-cell function compared with placebo (59), and the beneficial effects appear to be maintained, with patients showing significant improvements in glycemic control and a trend toward weight reduction after 12 weeks, as compared with sulfonyurea treatment (60). Mild initial and transient nausea/vomiting was reported, but otherwise no serious adverse side effects were noted.

Other approaches involving covalent binding to albumin (e.g., CJC-1131), resulting in a plasma elimination half-life of around 2 weeks in humans (corresponding to the circulating half-life of albumin itself), have also recently been reported (61). CJC-1131 lowers blood glucose in diabetic mice, and the effect persists up to 1 week following discontinuation of treatment (62).

From the available data, protease-resistant GLP-1 analogs appear to be associated with remarkably few undesirable side effects. Nausea and vomiting seem to be the most commonly reported adverse reaction, as might be expected from compounds based on a naturally occurring peptide with known gastrointestinal effects. However, it is noteworthy that even this symptom is generally reported as being transient, occurring primarily during the first week and then disappearing, suggesting that tachyphylaxis to the gastrointestinal effects may occur. Importantly, no severe hypoglycemic events have been reported. In the 5-day study with exenatide as monotherapy, no hypoglycemic events were reported (49), while after 1 month, only 9 (of >2,000) measurements revealed blood glucose levels of 3.6 mmol/l or less (50). There were no cases of severe hypoglycemia during 12 weeks of liraglutide monotherapy, and only 1 patient (of 135) reported minor hypoglycemia (60). In a study specifically designed to address this question, liraglutide was demonstrated not to impair glucagon-mediated hypoglycemia counterregulation (63). Even when exenatide was combined with patients' existing OAAs, those taking exenatide and metformin reported no hypoglycemic events (blood glucose <3.3 mmol/l), and although some (~19%) receiving exenatide and a sulfonylurea (with or without metformin) experienced mild-to-moderate hypoglycemia, none had severe hypoglyemia (51).

Enzyme inhibitors. The alternative approach, inhibiting degradation of endogenous GLP-1, has also been the focus of much interest. In particular, with the finding that GLP-1 is uniquely sensitive to DPP-IV cleavage in vivo, development of selective compounds to inhibit DPP-IV activity (thereby enhancing biologically active incretin concentrations) has been undertaken by a number of pharmaceutical companies, and several potent orally active DPP-IV inhibitors have been described. The use of such compounds has allowed substantiation of our initial hypothesis that DPP-IV inhibition may influence GLP-1 metabolism in vivo and lead to improvements in glucose tolerance (34). Thus, we demonstrated that the prototypal DPP-IV inhibitor, valine-pyrrolidide, eliminated NH_2-terminal degradation of GLP-1 in vivo, improving the metabolic stability of the intact biologically active peptide and potentiating its insulinotropic and antihyperglycemic effects in anesthetized pigs (64), whereas Pederson et al. (65) reported that another inhibitor, isoleucine-thiazolidide, improved glucose tolerance in rats. Subsequently, these results were corroborated in acute studies demonstrating that DPP-IV inhibition is effective in animal models of impaired glucose

tolerance (66,67). The mechanism of action appears to involve enhancement of endogenous, intact, biologically active GLP-1, because these levels increase following DPP-IV inhibition (66,67). However, valine-pyrrolidide also improves glucose tolerance in mice lacking the GLP-1 receptor (68), suggesting that DPP-IV inhibition may affect other substrates involved in glucose homeostasis. GIP is also a DPP-IV substrate (28,33), and DPP-IV inhibition reduces degradation of exogenous GIP, enhancing its insulinotropic and antihyperglycemic effects in anesthetized pigs (69), and increases intact endogenous GIP concentrations in conscious dogs (70), suggesting that preservation of intact GIP is likely to contribute to the improved glucose tolerance seen after DPP-IV inhibition. Indeed, after acute DPP-IV inhibition, it appears that all the beneficial effects on glucose tolerance are mediated via GLP-1 and GIP receptor signaling, since the glucose-lowering actions of DPP-IV inhibitors were eliminated in the double incretin receptor knockout (DIRKO) mouse (71), although it remains unknown whether other substrates may contribute after longer-term DPP-IV inhibition.

Data describing effects of long-term DPP-IV inhibition are now also available. In a 12-week study in Vancouver Zucker diabetic fatty (ZDF) rats, chronic DPP-IV inhibition with isoleucine-thiazolidide was associated with sustained improvements in glucose tolerance and β-cell responsiveness, which appeared to improve with time, and interestingly, by the end of the study, inhibitor-treated animals had lower body weights (72). Moreover, the same authors also demonstrated that chronic DPP-IV inhibition improves not only β-cell function, but also both hepatic and peripheral insulin sensitivity (73). The longer-acting inhibitor, FE 999-011, given twice daily, continuously inhibits plasma DPP-IV activity and was found to normalize the glucose excursion after oral glucose administration in Zucker obese rats (74). In ZDF rats, this compound actually delayed the onset of hyperglycemia and restored food and water intake to pre-diabetic levels. Active GLP-1 and pancreatic GLP-1 receptor mRNA levels were increased, suggesting the possibility that the inhibitor led to a GLP-1–mediated improvement in β-cell function (74). Together with other studies demonstrating that DPP-IV inhibition preserves islet function in diabetic mice (75) and improves β-cell survival and islet cell neogenesis in streptozotocin-induced diabetic rats (76), these results support the suggestion that DPP-IV inhibition may be able to prevent the transition from impaired glucose tolerance to overt type 2 diabetes (38).

In human studies, single doses of a DPP-IV inhibitor reduce the glucose excursion in healthy and diabetic subjects (77,78). The first chronic study, with two or three times daily administration of the short-acting inhibitor, NVP DPP728, to patients with mild type 2 diabetes gave clinical proof of the concept that DPP-IV inhibition is a viable approach to treating diabetes. Fasting and postprandial glucose concentrations were significantly reduced, and HbA_{1c} levels were lowered compared with placebo, even after only 4 weeks of treatment (79). NVP DPP728 was well tolerated, with only minor adverse events, including pruritis and nasopharyngitis, being reported; these adverse effects were described as being short lived and transient and did not lead to treatment being discontinued. Moreover, they appear to be drug specific and unrelated to DPP-IV inhibition per se, since similar symptoms were not reported for another inhibitor, LAF237, which has now reached phase 3 clinical development (80). LAF237 is longer acting than NVP DPP728, and once-daily treatment for 4 weeks significantly improves metabolic control. Fasting and postprandial glucose concentrations and HbA_{1c} levels were significantly reduced compared with placebo, insulin secretion was sustained, and postprandial levels of active GLP-1 were increased. Moreover, glucagon concentrations were significantly reduced by LAF237 (80), suggesting that GLP-1–mediated inhibition of glucagon secretion, in addition to its insulinotropic effects, contributes to mediating the effects of DPP-IV inhibition. To date, there are no reports of changes in body weight in humans after DPP-IV inhibitor treatment.

There has been some debate over whether DPP-IV inhibitor monotherapy will be as effective as GLP-1 receptor agonist therapy and indeed whether it will be effective enough to be clinically useful at all. This was largely based on the assumption that the mechanism of action of DPP-IV inhibitors was predominately reliant on preventing degradation of endogenous GLP-1, raising the question of whether this would be sufficient to have a significant effect in type 2 diabetes. However, it is now clear that in addition to GLP-1, intact (active) endogenous GIP levels are also enhanced (70) and glucagon levels are lowered (80), although whether this is secondary to increased GLP-1 is unclear. It therefore seems likely that DPP-IV inhibitors exert their beneficial effects on glucose tolerance via effects on several different endogenous substrates. Preclinical studies indicate that DPP-IV inhibitors do have positive effects on glucose tolerance in animal models of diabetes, and the only chronic studies in humans reported so far have also yielded promising results, suggesting that DPP-IV inhibitor monotherapy is a feasible treatment option. How DPP-IV inhibitors will compare with GLP-1 receptor agonists in terms of efficacy is, as yet, unknown, and direct comparison in matched patient groups will be required before this question can be answered.

The limited human data suggest that DPP-IV inhibitors are well tolerated, and DPP-IV inhibition does not seem to be associated with hypoglycemic events. Four (of 61) patients treated with NVP DPP728 for 28 days reported symptoms suggestive of hypoglycemia, but only 1 had a blood glucose level of <3.3 mmol/l (79), whereas no hypoglycemic incidences were reported for LAF237 (80). Preliminary studies with LAF237 indicate that it does not significantly increase the risk for hypoglycemia when given together with the sulfonylurea, glibenclamide (81).

The possibility of other side effects unrelated to incretin hormone metabolism has also been the subject of some concern. The incretin hormones are not the only substrates for DPP-IV, raising the possibility that inhibition of the cleavage of other endogenous DPP-IV substrates may give rise to undesirable side effects. Among the additional substrates identified in kinetic studies are a number of neuropeptides, including pituitary adenylylate cyclase–activating polpeptide (PACAP), vasoactive intestinal polypeptide (VIP), gastrin-releasing peptide (GRP), neuropeptide Y (NPY), and growth hormone–releasing hormone (GHRH), other regulatory peptides (such as GLP-2

and peptide YY [PYY]), as well as a number of chemokines and cytokines (rev. in 82). However, it should be noted that it is unknown how many of the potential substrates identified in kinetic studies are actually endogenous substrates in vivo or moreover whether DPP-IV is the major mediator of their elimination or whether they are metabolized by other enzymes. DPP-IV is also found as a membrane-associated molecule on the surface of T-cells (where it is known as CD26). Here, it contributes to T-cell activation and proliferation via its interaction with other membrane-expressed antigens such as CD45 (83), raising the possibility that DPP-IV inhibition may compromise immune function, although it is unclear whether the catalytic activity per se is required for CD26's immune role or even whether its role is essential. In these contexts, it is relevant that both Fischer rats with mutations in the catalytic site and mice with a targeted deletion of the gene encoding CD26 are completely viable and seem to suffer no ill effects because of the lack of DPP-IV (33,68). Furthermore, no adverse side effects were reported during chronic DPP-IV inhibition in rodents (72–76), while early indications from the 4-week clinical trials also show good tolerability with few adverse events (79,80), suggesting that DPP-IV inhibition may be a safe and effective treatment, although longer-term studies are needed to confirm this.

As discussed above, other enzymes may additionally be involved in determining the metabolic stability of GLP-1; Hupe-Sodmann et al. (36,37) demonstrated that GLP-1 is a substrate for NEP 24.11 in vitro. Studies from the author's laboratory have indicated that NEP 24.11 may indeed have a physiological role in GLP-1 metabolism, because the selective NEP 24.11 inhibitor, candoxatril, increases the plasma half-life of GLP-1. By itself, this has only a modest effect in potentiating the antihyperglycemic effect of exogenous GLP-1, resulting in a small reduction in the glucose excursion following intravenous glucose in anesthetized pigs, presumably because GLP-1 is still susceptible to NH_2-terminal truncation by DPP-IV (A. Plamboeck and C.F.D., unpublished observations). However, when DPP-IV and NEP 24.11 inhibitors are administered concomitantly, the combined effect is greater than the effect of either inhibitor alone, resulting in significant improvements in the antihyperglycemic and insulinotropic effects of exogenous GLP-1 (84). Results of studies demonstrating effects on endogenous GLP-1 concentrations together with potential effects on glucose tolerance are awaited. Of interest, the first preliminary report of a compound possessing potent dual DPP-IV and NEP 24.11 inhibitory activity has recently been presented (85).

CONCLUSIONS

The studies discussed above support the idea that a GLP-1–based therapy will be a safe and effective treatment for type 2 diabetes. The clinical studies reported so far indicate that this approach, whether achieved by DPP-IV inhibition or by GLP-1 receptor agonists, has the potential to reduce and maybe even normalize both fasting and postprandial glucose concentrations, without having an adverse effect on weight gain. Moreover, the preclinical studies raise the hope that such a therapy may be able to delay or even halt the progression of the disease, or possibly even prevent its development, by providing a means of safely treating subjects with impaired glucose tolerance. Finally, but by no means least, this approach may turn out to be inherently safer than existing insulin secretagogues, because of its glucose dependency. Thus, GLP-1 receptor agonists and DPP-IV inhibitors have not been associated with any incidences of severe hypoglycemia, even when given in combination with existing OAAs, while when given as monotherapy, virtually no hypoglycemic events have been reported.

Although the two approaches (GLP-1 receptor agonists and DPP-IV inhibitors) can be described as being "GLP-1 based," there are clear differences between them, the most obvious of which is their route of administration. The GLP-1 receptor agonists described so far are all based on the native peptide, meaning that they are not orally available, whereas DPP-IV inhibitors are low–molecular weight compounds suitable for oral administration. However, future developments may provide alternative means of administration of GLP-1 analogs, in analogy with the possibility of intrapulmonary administration or buccal and skin uptake of insulin (rev. in 86). In this context, buccal absorption of native GLP-1, resulting in blood glucose reductions in diabetic patients, has been described (87). However, other differences mean that DPP-IV inhibitors cannot be regarded as being an "oral GLP-1." GLP-1 analogs, by virtue of their enhanced plasma survival time, have kinetic profiles that elevate plasma levels into the therapeutic range for prolonged periods, giving 24-h antihyperglycemic coverage, while DPP-IV inhibitors are likely to potentiate the natural diurnal rhythms of their substrates (e.g., enhancing meal-stimulated intact incretin levels). Secondly, the dose of the GLP-1 analogs can be titrated according to the patient's need, whereas DPP-IV inhibition only preserves the endogenously secreted peptide from degradation, meaning there is a limit to how far plasma levels of the active peptide can increase, although it might be possible to combine a DPP-IV inhibitor with a GLP-1 secretagogue in order to raise GLP-1 levels further. Thirdly, all of the effects of the analogs are mediated via the GLP-1 receptor, while emerging data suggest that DPP-IV inhibitors are likely to be multifactorial in their mechanism of action. Finally, and in contrast to OAAs like sulfonylureas, chronic treatment of type 2 diabetic patients with native GLP-1 (18) and the GLP-1 analogs (52,60) seems to be associated with beneficial body weight reductions, whereas until longer-term clinical studies are reported, it is unknown how DPP-IV inhibitors will fare in this respect. Only direct comparison in the clinical setting will reveal how (or whether) these differences between the two approaches will *1*) affect their ability to treat the symptoms of the diabetic phenotype effectively and safely and *2*) show which patient groups are most likely to benefit. In the meantime, both GLP-1 receptor agonists and DPP-IV inhibitors represent promising new approaches to therapy of type 2 diabetes.

NOTE ADDED IN PROOF

At the recent 64th Scientific Sessions of the American Diabetes Association, Orlando, Florida, 4–8 June 2004, encouraging preliminary clinical results with LAF237 as 12 weeks' monotherapy and for 1 year in combination with metformin were presented [Pratley R, Galbreath E:

Twelve-week monotherapy with the DPP-4 inhibitor, LAF237 improves glycemic control in patients with type 2 diabetes (T2DM) (Abstract). *Diabetes* 53 (Suppl. 2):A83, 2004; Ahrén B, Gomis R, Standl E, Mills D, Schweizer A: Prolonged efficacy of LAF237 in patients with type 2 diabetes (T2DM) inadequately controlled with metformin (Abstract). Late-breaking abstract 7-LB]. At the same meeting, preclinical data suggested that inhibition of DPP-8 and/or DPP-9 gave rise to some toxicological signs and immunological effects [Leiting B, Nichols E, Biftu T, Edmonsen S, Ok H, Weber AE, Zaller D, Thornberry NA: Inhibition of dipeptidyl peptidase IV does not attenuate T cell activation in vitro (Abstract). *Diabetes* 53 (Suppl. 2):A2, 2004; Lankas G, Leiting B, Roy RS, Eierman G, Biftu T, Kim D, Ok H, Weber AE, Thornberry NA: Inhibition of DPP8/9 results in toxicity in preclinical species: potential importance of selective dipeptidyl peptidase IV inhibition for the treatment of type 2 DM (Abstract). *Diabetes* 53 (Suppl. 2):A2, 2004], indicating that selectivity for DPP-IV versus other related enzymes may be of considerable relevance with respect to their therapeutic application.

REFERENCES

1. Bell GI, Santerre RF, Mullenbach GT: Hamster preproglucagon contains the sequence of glucagon and two related peptides. *Nature* 302:716–718, 1983
2. Ørskov C, Holst JJ, Knuhtsen S, Baldissera FG, Poulsen SS, Nielsen OV: Glucagon-like peptides GLP-1 and GLP-2, predicted products of the proglucagon gene, are secreted separately from pig small intestine but not pancreas. *Endocrinology* 119:1467–1475, 1986
3. Mojsov S, Weir GC, Habener JF: Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J Clin Invest* 79:616–619, 1987
4. Holst JJ, Ørskov C, Nielsen OV, Schwartz TW: Truncated glucagon-like peptide 1, an insulin-releasing hormone from the distal gut. *FEBS Lett* 211:169–174, 1987
5. Holst JJ: Therapy of type 2 diabetes mellitus based on the actions of glucagon-like peptide-1. *Diabetes Metab Res Rev* 18:430–441, 2002
6. Fehmann HC, Habener JF: Insulinotropic hormone glucagon-like peptide-I(7-37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma beta TC-1 cells. *Endocrinology* 130:159–166, 1992
7. Xu G, Stoffers DA, Habener JF, Bonner-Weir S: Exendin-4 stimulates both β-cell replication and neogenesis, resulting in increased β-cell mass and improved glucose tolerance in diabetic rats. *Diabetes* 48:2270–2276, 1999
8. Stoffers DA, Kieffer TJ, Hussain MA, Drucker DJ, Bonner-Weir S, Habener JF, Egan JM: Insulinotropic glucagon-like peptide 1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in mouse pancreas. *Diabetes* 49:741–748, 2000
9. Zhou J, Wang X, Pineyro MA, Egan JM: Glucagon-like peptide 1 and exendin-4 convert pancreatic AR42J cells into glucagon- and insulin-producing cells. *Diabetes* 48:2358–2366, 1999
10. Perfetti R, Zhou J, Doyle ME, Egan JM: Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. *Endocrinology* 141:4600–4605, 2000
11. Farilla L, Bulotta A, Hirshberg B, Li Calzi S, Khoury N, Noushmehr H, Bertolotto C, Di Mario U, Harlan DM, Perfetti R: Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. *Endocrinology* 144:5149–5158, 2003
12. Li Y, Hansotia T, Yusta B, Ris F, Halban PA, Drucker DJ: Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. *J Biol Chem* 278:471–478, 2003
13. Wang Q, Li L, Xu E, Wong V, Rhodes C, Brubaker P: Glucagon-like peptide-1 regulates proliferation and apoptosis via activation of protein kinase B in pancreatic INS-1 beta cells. *Diabetologia* 47:478–487, 2004
14. Johnson JD, Han Z, Otani K, Ye H, Zhang Y, Wu H, Horikawa Y, Misler S, Bell GI, Polonsky KS: RyR2 and calpain-10 delineate a novel apoptosis pathway in pancreatic islets. *J Biol Chem* [Epub ahead of print], 25 March 2004
15. Nauck MA, Heimesaat MM, Behle K, Holst JJ, Nauck MS, Ritzel R, Hufner M, Schmiegel WH: Effects of glucagon-like peptide 1 on counterregulatory hormone responses, cognitive functions, and insulin secretion during hyperinsulinemic, stepped hypoglycemic clamp experiments in healthy volunteers. *J Clin Endocrinol Metab* 87:1239–1246, 2002
16. Nauck MA, Niedereichholz U, Ettler R, Holst JJ, Orskov C, Ritzel R, Schmiegel WH: Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. *Am J Physiol* 273:E981–E988, 1997
17. Verdich C, Flint A, Gutzwiller JP, Naslund E, Beglinger C, Hellstrom PM, Long SJ, Morgan LM, Holst JJ, Astrup A: A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on ad libitum energy intake in humans. *J Clin Endocrinol Metab* 86:4382–4389, 2001
18. Zander M, Madsbad S, Madsen JL, Holst JJ: Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet* 359:824–830, 2002
19. Gutzwiller JP, Goke B, Drewe J, Hildebrand P, Ketterer S, Handschin D, Winterhalder R, Conen D, Beglinger C: Glucagon-like peptide-1: a potent regulator of food intake in humans. *Gut* 44:81–86, 1999
20. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JP, Smith DM, Ghatei MA, Herbert J, Bloom SR: A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 379:69–72, 1996
21. Ørskov C, Poulsen SS, Moller M, Holst JJ: Glucagon-like peptide I receptors in the subfornical organ and the area postrema are accessible to circulating glucagon-like peptide I. *Diabetes* 45:832–835, 1996
22. Vrang N, Phifer CB, Corkern MM, Berthoud HR: Gastric distension induces c-Fos in medullary GLP-1/2-containing neurons. *Am J Physiol* 285:R470–R478, 2003
23. Nauck M, Stockmann F, Ebert R, Creutzfeldt W: Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. *Diabetologia* 29:46–52, 1986
24. Vilsbøll T, Krarup T, Deacon CF, Madsbad S, Holst JJ: Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* 50:609–613, 2001
25. Nauck MA, Kleine N, Ørskov C, Holst JJ, Willms B, Creutzfeldt W: Normalization of fasting hyperglycemia by exogenous GLP-1 (7-36 amide) in type 2 diabetic patients. *Diabetologia* 36:741–744, 1993
26. Nauck MA, Sauerwald A, Ritzel R, Holst JJ, Schmiegel W: Influence of glucagon-like peptide 1 on fasting glycemia in type 2 diabetic patients treated with insulin after sulfonylurea secondary failure. *Diabetes Care* 21:1925–1931, 1998
27. Nauck MA, Wollschläger D, Werner J, Holst JJ, Ørskov C, Creutzfeldt W, Willms B: Effects of subcutaneous glucagon-like peptide 1 (GLP-1 [7-36 amide]) in patients with NIDDM. *Diabetologia* 39:1546–1553, 1996
28. Mentlein R, Gallwitz B, Schmidt WE: Dipeptidyl peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1 (7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur J Biochem* 214:829–835, 1993
29. Knudsen LB, Pridal, L: Glucagon-like peptide-1 (9-36)amide is a major metabolite of glucagon-like peptide-1 (7-36)amide after in vivo administration to dogs, and it acts as an antagonist on the pancreatic receptor. *Eur J Pharmacol* 318:429–435, 1996
30. Deacon CF, Plamboeck A, Moller S, Holst JJ: GLP-1-(9-36) amide reduces blood glucose in anesthetized pigs by a mechanism that does not involve insulin secretion. *Am J Physiol* 282:E873–E879, 2002
31. Vahl TP, Paty BW, Fuller BD, Prigeon RL, D'Alessio DA: Effects of GLP-1-(7-36)NH2, GLP-1-(7-37), and GLP-1-(9-36)NH2 on intravenous glucose tolerance and glucose-induced insulin secretion in healthy humans. *J Clin Endocrinol Metab* 88:1772–1779, 2003
32. Deacon CF, Johnsen AH, Holst, JJ: Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide which is a major endogenous metabolite in vivo. *J Clin Endocrinol Metab* 80:952–957, 1995
33. Kieffer TJ, McIntosh CHS, Pederson RA: Degradation of glucose-dependent insulinotropic polypeptide (GIP) and truncated glucagon-like peptide 1 (tGLP-1) in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology* 136:3585–3596, 1995
34. Deacon CF, Nauck MA, Toft-Nielsen M, Pridal L, Willms B, Holst JJ: Both subcutaneously and intravenously administered glucagon-like peptide-1 are rapidly degraded from the NH_2-terminus in type II diabetic patients and in healthy subjects. *Diabetes* 44:1126–1131, 1995
35. Hansen L, Deacon CF, Ørskov C, Holst JJ: Glucagon-like peptide-1-(7-36)amide is transformed to glucagon-like peptide-1-(9-36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. *Endocrinology* 140:5356–5363, 1999
36. Hupe-Sodmann K, McGregor GP, Bridenbaugh R, Goke R, Goke B, Thole H, Zimmermann B, Voigt K: Characterisation of the processing by human

neutral endopeptidase 24.11 of GLP-1(7-36) amide and comparison of the substrate specificity of the enzyme for other glucagon-like peptides. *Regul Pept* 58:149–156, 1995
37. Hupe-Sodmann K, Goke R, Goke B, Thole HH, Zimmermann B, Voigt K, McGregor GP: Endoproteolysis of glucagon-like peptide (GLP)-1 (7-36) amide by ectopeptidases in RINm5F cells. *Peptides* 18:625–632, 1997
38. Holst JJ, Deacon CF: Inhibition of the activity of dipeptidyl peptidase IV as a treatment for type 2 diabetes. *Diabetes* 47:1663–1670, 1998
39. Deacon CF, Knudsen LB, Madsen K, Wiberg FC, Jacobsen O, Holst JJ: Dipeptidyl peptidase IV resistant analogues of glucagon-like peptide-1 which have extended metabolic stability and improved biological activity. *Diabetologia* 41:271–278, 1998
40. Burcelin R, Dolci W, Thorens B: Long-lasting antidiabetic effect of a dipeptidyl peptidase IV-resistant analog of glucagon-like peptide-1. *Metabolism* 48:252–258, 1999
41. Edwards CM, Stanley SA, Davis R, Brynes AE, Frost GS, Seal LJ, Ghatei MA, Bloom SR: Exendin-4 reduces fasting and postprandial glucose and decreases energy intake in healthy volunteers. *Am J Physiol* 281:E155–E161, 2001
42. Vilsbøll T, Agerso H, Krarup T, Holst JJ: Similar elimination rates of glucagon-like peptide-1 in obese type 2 diabetic patients and healthy subjects. *J Clin Endocrinol Metab* 88:220–224, 2003
43. Greig NH, Holloway HW, De Ore KA, Jani D, Wang Y, Zhou J, Garant MJ, Egan JM: 1999 Once daily injection of exendin-4 to diabetic mice achieves long-term beneficial effects on blood glucose concentrations. *Diabetologia* 42:45–50, 1999
44. Szayna M, Doyle ME, Betkey JA, Holloway HW, Spencer RG, Greig NH, Egan JM: Exendin-4 decelerates food intake, weight gain, and fat deposition in Zucker rats. *Endocrinology* 141:1936–1941, 2000
45. Tourrel C, Bailbe D, Lacorne M, Meile MJ, Kergoat M, Portha B: Persistent improvement of type 2 diabetes in the Goto-Kakizaki rat model by expansion of the β-cell mass during the prediabetic period with glucagon-like peptide-1 or exendin-4. *Diabetes* 51:1443–1452, 2002
46. Wang Q, Brubaker PL: Glucagon-like peptide-1 treatment delays the onset of diabetes in 8 week-old db/db mice. *Diabetologia* 45:1263–1273, 2002
47. Tourrel C, Bailbe D, Meile MJ, Kergoat M, Portha B: Glucagon-like peptide-1 and exendin-4 stimulate β-cell neogenesis in streptozotocin-treated newborn rats resulting in persistently improved glucose homeostasis at adult age. *Diabetes* 50:1562–1570, 2001
48. Stoffers DA, Desai BM, DeLeon DD, Simmons RA: Neonatal exendin-4 prevents the development of diabetes in the intrauterine growth retarded rat. *Diabetes* 52:734–740, 2003
49. Kolterman OG, Buse JB, Fineman MS, Gaines E, Heintz S, Bicsak TA, Taylor K, Kim D, Aisporna M, Wang Y, Baron AD: Synthetic exendin-4 (exenatide) significantly reduces postprandial and fasting plasma glucose in subjects with type 2 diabetes. *J Clin Endocrinol Metab* 88:3082–3089, 2003
50. Egan JM, Meneilly GS, Elahi D: Effects of 1-mo bolus subcutaneous administration of exendin-4 in type 2 diabetes. *Am J Physiol* 284:E1072–E1079, 2003
51. Fineman MS, Bicsak TA, Shen LZ, Taylor K, Gaines E, Varns A, Kim D, Baron AD: Effect on glycemic control of exenatide (synthetic exendin-4) additive to existing metformin and/or sulfonlyurea treatment in patients with type 2 diabetes. *Diabetes Care* 26:2370–2377, 2003
52. Baron A, Poon T, Taylor K, Nielsen L, Boies S, Zhou J, Zhuang D, Varns A, Kim D, Fineman M, Kolterman O: Exenatide (synthetic extendin-4) showed marked HbA_{1c} decline over 5 months in patients with type 2 diabetes failing oral agents in an open-label study (Abstract). Presented at the *63rd Scientific Sessions of the American Diabetes Association, New Orleans, LA, 13–17 June 2003* (Late-breaking abstract 3-LB)
53. Trautmann ME, Chen CF, Chappell J, Danaberg J, Patterson B, Klausmann G, Kapitza C: LY307161 SR, a long-scting GLP-1 analog, improved glycemic control in patients with type 2 diabetes (Abstract). *Diabetes* 52 (Suppl. 1):A136, 2003
54. Knudsen LB, Nielsen PF, Huusfeldt PO, Johansen NL, Madsen K, Pedersen FZ, Thogersen H, Wilken M, Agerso H: Potent derivatives of glucagon-like peptide-1 with pharmacokinetic properties suitable for once daily administration. *J Med Chem* 43:1664–1669, 2002
55. Juhl CB, Hollingdal M, Sturis J, Jakobsen G, Agerso H, Veldhuis J, Porksen N, Schmitz O: Bedtime administration of NN2211, a long-acting GLP-1 derivative, substantially reduces fasting and postprandial glycemia in type 2 diabetes. *Diabetes* 51:424–429, 2002
56. Rolin B, Larsen MO, Gotfredsen CF, Deacon CF, Carr RD, Wilken M, Knudsen LB: The long-acting GLP-1 derivative NN2211 ameliorates glycemia and increases beta-cell mass in diabetic mice. *Am J Physiol* 283:E745–E752, 2002
57. Sturis J, Gotfredsen CF, Romer J, Rolin B, Ribel U, Brand CL, Wilken M, Wassermann K, Deacon CF, Carr RD, Knudsen LB: GLP-1 derivative liraglutide in rats with beta-cell deficiencies: influence of metabolic state on beta-cell mass dynamics. *Br J Pharmacol* 140:123–132, 2003
58. Chang AM, Jakobsen G, Sturis J, Smith MJ, Bloem CJ, An B, Galecki A, Halter JB: The GLP-1 derivative NN2211 restores β-cell sensitivity to glucose in type 2 diabetic patients after a single dose. *Diabetes* 52:1786–1791, 2003
59. Degn KB, Juhl CB, Sturis J, Jakobsen G, Brock B, Chandramouli V, Rungby J, Landau BR, Schmitz O: One week's treatment with the long-acting glucagon-like peptide 1 derivative liraglutide (NN2211) markedly improves 24-h glycemia and α- and β-cell function and reduces endogenous glucose release in patients with type 2 diabetes. *Diabetes* 53:1187–1194, 2004
60. Madsbad S, Schmitz O, Ranstam J, Jakobsen G, Matthews DR: Improved glycemic control with no weight incease in patients with type 2 diabetes after once-daily treatment with the long-acting glucagon-like peptide 1 analog liraglutide (NN2211): a 12-week, double-blind, randomized, controlled trial. *Diabetes Care* 27:1335–1342, 2004
61. Lawrence B, Drefus JF, Wen S, Guivarc'h PH, Drucker DJ, Castaigne JP: CJC-1131, a long-scting GLP-1 derivative, exhibits an extended pharmacokinetic profile in healthy human volunteers (Abstract). *Diabetes* 52 (Suppl. 1):A125, 2003
62. Kim JG, Baggio LL, Bridon DP, Castaigne JP, Robitaille MF, Jette L, Benquet C, Drucker DJ: Development and characterization of a glucagon-like peptide 1-albumin conjugate: the ability to activate the glucagon-like peptide 1 receptor in vivo. *Diabetes* 52:751–759, 2003
63. Nauck MA, El-Ouaghlidi, Hompesch M, Jacobsen J, Elbroend B: No impairment of hypoglycemia counterregulation via glucagon with the long-acting GLP-1 derivative, NN2211, in subjects with type 2-diabetes (Abstract). *Diabetologia* 46 (Suppl. 2):A285, 2003
64. Deacon CF, Hughes TE, Holst JJ: Dipeptidyl peptidase IV inhibition potentiates the insulinotropic effect of glucagon-like peptide-1 in anesthetized pigs. *Diabetes* 47:764–769, 1998
65. Pederson RA, White HA, Schlenzig D, Pauly RP, McIntosh CH, Demuth HU: Improved glucose tolerance in Zucker fatty rats by oral administration of the dipeptidyl peptidase IV inhibitor isuleucine thiazolidide. *Diabetes* 47:1253–1258, 1998
66. Balkan B, Kwasnik L, Miserendino R, Holst JJ, Li X: Inhibition of dipeptidyl peptidase IV with NVP-DPP728 increases plasma GLP-1 (7-36 amide) concentrations and improves oral glucose tolerance in obese Zucker rats. *Diabetologia* 42:1324–1331, 1999
67. Ahrén B, Holst JJ, Martensson H, Balkan B: Improved glucose tolerance and insulin secretion by inhibition of dipeptidyl peptidase IV in mice. *Eur J Pharmacol* 404:239–245, 2000
68. Marguet D, Baggio L, Kobayashi T, Bernard AM, Pierres M, Nielsen PF, Ribel U, Watanabe T, Drucker DJ, Wagtmann N: Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proc Natl Acad Sci U S A* 97:6874–6879, 2000
69. Deacon CF, Danielsen P, Klarskov L, Olesen M, Holst JJ: Dipeptidyl peptidase IV inhibition reduces the degradation and clearance of GIP and potentiates its insulinotropic and antihyperglycemic effects in anesthetized pigs. *Diabetes* 50:1588–1597, 2001
70. Deacon CF, Wamberg S, Bie P, Hughes TE, Holst JJ: Preservation of active incretin hormones by inhibition of dipeptidyl peptidase IV suppresses meal-induced incretin secretion in dogs. *J Endocrinol* 172:355–362, 2002
71. Hansotia T, Baggio LL, Delmeire D, Hinke SA, Yamada Y, Tsukiyama K, Seino Y, Holst JJ, Schuit F, Drucker DJ: Double incretin receptor knockout (DIRKO) mice reveal an essential role for the enteroinsular axis in transducing the glucoregulatory actions of DPP-IV inhibitors. *Diabetes* 53:1326–1335, 2004
72. Pospisilik JA, Stafford SG, Demuth HU, Brownsey R, Parkhouse W, Finegood DT, McIntosh CHS, Pederson RA: Long-term treatment with the dipeptidyl peptidase IV inhibitor P32/98 causes sustained improvements in glucose tolerance, insulin sensitivity, hyperinsulinemia, and β-cell glucose responsiveness in VDF *(fa/fa)* Zucker rats. *Diabetes* 51:943–950, 2002
73. Pospisilik JA, Stafford SG, Demuth HU, McIntosh CH, Pederson RA: Long-term treatment with dipeptidyl peptidase IV inhibitor improves hepatic and peripheral insulin sensitivity in the VDF Zucker rat: a euglycemic-hyperinsulinemic clamp study. *Diabetes* 51:2677–2683, 2002
74. Sudre B, Broqua P, White RB, Ashworth D, Evans DM, Haigh R, Junien JL, Aubert ML: Chronic inhibition of circulating dipeptidyl peptidase IV by FE 999011 delays the occurrence of diabetes in male zucker diabetic fatty rats. *Diabetes* 51:1461–1469, 2002
75. Reimer MK, Holst JJ, Ahrén B: Long-term inhibition of dipeptidyl peptidase IV improves glucose tolerance and preserves islet function in mice. *Eur J Endocrinol* 146:717–727, 2002
76. Pospisilik JA, Martin J, Doty T, Ehses JA, Pamir N, Lynn FC, Piteau S, Demuth HU, McIntosh CH, Pederson RA: Dipeptidyl peptidase IV inhibitor

treatment stimulates β-cell survival and islet neogenesis in streptozotocin-induced diabetic rats. *Diabetes* 52:741–50, 2003

77. Demuth HU, Hoffmann T, Glund K, McIntosh CHS, Pederson RA, Fueker K, Fischer S, Hanefeld M: Single dose treatment of diabetic patients by the DP IV inhibitor P32/98 (Abstract). *Diabetes* 49 (Suppl. 1):A102, 2002
78. Rothenburg P, Kalbag J, Smith H, Gingerich R, Nedelman J, Villhauer E, McLeod J, Hughes T: Treatment with a DPP-IV inhibitor, NVP-DPP728, increases prandial intact GLP-1 levels and reduces glucose exposure in humans (Abstract). *Diabetes* 49 (Suppl. 1):A39, 2000
79. Ahrén B, Simonsson E, Larsson H, Landin-Olsson M, Torgeirsson H, Jansson PA, Sandqvist M, Bavenholm P, Efendic S, Eriksson JW, Dickinson S, Holmes D: Inhibition of dipeptidyl peptidase IV improves metabolic control over a 4-week study period in type 2 diabetes. *Diabetes Care* 25:869–875, 2002
80. Ahrén B, Landin-Olsson M, Jansson PA, Svensson M, Holmes D, Schweizer A: Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. *J Clin Endocrinol Metab* 89:2078–2084, 2004
81. El-Ouaghlidi A, Rehring ER, Schweizer A, Holmes D, Nauck MA: The dipeptidyl peptidase IV inhibitor, LAF237 does not accentuate reactive hypoglycaemia caused by the sulphonylurea glibenclamide administered before an oral glucose load in healthy subjects (Abstract). *Diabetes* 52 (Suppl. 1):A118, 2003
82. Lambeir AM, Durinx C, Scharpe S, De Meester I: Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions, and clinical aspects of the enzyme DPP IV. *Crit Rev Clin Lab Sci* 40:209–294, 2003.
83. Fleicher B: CD26: a surface protease involved in T-cell activation. *Immunol Today* 15:180–184, 1994
84. Plamboeck A, Holst JJ, Carr RD, Deacon CF: Neutral endopeptidase 24.11 and dipeptidyl peptidase IV are both involved in regulating the metabolic stability of glucagon-like peptide-1 in vivo. *Adv Exp Med Biol* 524:303–312, 2002
85. Oefner C, Pierau S, Dale GE: Ectopeptidases and type II diabetes (Abstract). Presented at the *11th Protein Structure Determination in Industry, Cambridge, U.K., 17–18 November 2003* (Abstract T001)
86. Owens DR, Zinman B, Bolli G: Alternative routes of insulin delivery. *Diabet Med* 20:886–898, 2003
87. Gutniak MK, Larsson H, Sanders SW, Juneskans O, Holst JJ, Ahren B: GLP-1 tablet in type 2 diabetes in fasting and postprandial conditions. *Diabetes Care* 20:1874–1879, 1997